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(54) Title: HUMAN SIGNAL PEPTIDE-CONTAINING PROTEINS

(57) Abstract

    The invention provides a human signal peptide-containing proteins (SIGP) and polynucleotides which identify and encode SIGP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of SIGP.

HUMAN SIGNAL PEPTIDE-CONTAINING PROTEINS

TECHNICAL FIELD

5   This invention relates to nucleic acid and amino acid sequences of human signal peptide-containing proteins and to the use of these sequences in the diagnosis, treatment, and prevention of cancer and immunological disorders.

BACKGROUND OF THE INVENTION

10   Protein transport is an essential process for all living cells. Transport of an individual protein usually occurs via an amino-terminal signal sequence which directs, or targets, the protein from its ribosomal assembly site to a particular cellular or extracellular location. Transport may involve any combination of several of the following steps: contact with a chaperone, unfolding, interaction with a receptor and/or a pore complex, addition of

15   energy, and refolding. Moreover, an extracellular protein may be produced as an inactive precursor. Once the precursor has been exported, removal of the signal sequence by a signal peptidase and posttranslational processing (e.g., glycosylation or phosphorylation) activates the protein. Signal sequences are common to receptors, matrix molecules (e.g., adhesion, cadherin, extracellular matrix, integrin, and selectin), cytokines, hormones,

20   growth and differentiation factors, neuropeptides, vasomediators, phosphokinases, phosphatases, phospholipases, phosphodiesterases, G and Ras-related proteins, ion channels, transporters/pumps, proteases, and transcription factors.

         G-protein coupled receptors (GPCRs) are a superfamily of integral membrane proteins which transduce extracellular signals. GPCRs include receptors for biogenic

25   amines, e.g., dopamine, epinephrine, histamine, glutamate (metabotropic effect), acetylcholine (muscarinic effect), and serotonin; for lipid mediators of inflammation such as prostaglandins, platelet activating factor, and leukotrienes; for peptide hormones such as calcitonin, C5a anaphylatoxin, follicle stimulating hormone, gonadotropin releasing hormone, neurokinin, oxytocin, and thrombin; and for sensory signal mediators, e.g.,

30   retinal photopigments and olfactory stimulatory molecules.

         The structure of these highly-conserved receptors consists of seven hydrophobic

transmembrane regions, cysteine disulfide bridges between the second and third

extracellular loops, an extracellular N-terminus, and a cytoplasmic C-terminus. Three

extracellular loops alternate with three intracellular loops to link the seven transmembrane

regions. The N-terminus interacts with ligands, the disulfide bridge interacts with agonists

5   and antagonists, and the large third intracellular loop interacts with G proteins to activate

second messengers such as cyclic AMP (cAMP), phospholipase C, inositol triphosphate,

or ion channel proteins. The most conserved parts of these proteins are the transmembrane

regions and the first two cytoplasmic loops. A conserved, acidic-Arg-aromatic triplet

present in the second cytoplasmic loop may interact with the G proteins. The consensus

10   pattern,

[GSTALIVMYWC]-[GSTANCPDE]-{EDPKRH}-x(2)-[LIVMNQGA]-x(2)-[LIVMFT]-[

GSTANC]-[LIVMFYWSTAC]-[DENH]- R-[FYWCSH]-x(2)-[LIVM] is characteristic of

most proteins belonging to this superfamily. (Watson, S. and Arkinstall, S. (1994) The G-

protein Linked Receptor Facts Book, Academic Press, San Diego, CA, pp. 2-6; and

15   Bolander, F.F. (1994) Molecular Endocrinology, Academic Press, San Diego, CA, pp. 8-

19.)

        Tetraspanins are a superfamily of membrane proteins which facilitate the formation

and stability of cell-surface signaling complexes containing lineage-specific proteins,

integrins, and other tetraspanins. They are involved in cell activation, proliferation

20   (including cancer), differentiation, adhesion, and motility. These proteins cross the

membrane four times, have conserved intracellular N- and C-termini and an extracellular,

non-conserved hydrophilic domain. Three highly conserved polar amino acids are located

in the transmembrane domains (TM), an asparagine in TM1 and a glutamate or glutamine

in TM3 and TM4. Two to three conserved charged residues, including a glutamic acid

25   residue, are present in the cytoplasmic loop between TM2 and TM3. The extracellular

loop between TM3 and TM4 contains four conserved cysteine residues: two in a conserved

CCG motif located about 50 residues C-terminal to TM3; one, often preceded by glycine,

11 residues N-terminal to TM4; and one in the extracellular loop may be found in a PXSC

motif. Tetraspanins include, e.g., platelet and endothelial cell membrane proteins,

30   leukocyte surface proteins, tissue specific and tumorous antigens, and the retinitis

pigmentosa-associated gene peripherin. (Maecker, H.T. et al. (1997) FASEB J. 11:428-

442.) Matrix proteins (Mps) function in formation, growth, remodeling and maintenance

of tissues and as important mediators and regulators of the inflammatory response. The

expression and balance of MPs may be perturbed by biochemical changes that result from

congenital, epigenetic, or infectious diseases. In addition, MPs affect leukocyte migration,

proliferation, differentiation, and activation in immune response.

5   MPs encompass a variety of proteins and their functions. Extracellular matrix

(ECM) proteins are multidomain proteins that play an important role in the diverse

functions of the ECM. ECM proteins are frequently characterized by the presence of one

or more domains which may include collagen-like domains, EGF-like domains,

immunoglobulin-like domains, fibronectin-like domains, vWFA-like modules. (Ayad, S.

10   et al. (1994) The Extracellular Matrix Facts Book, Academic Press, San Diego, CA, pp. 2-

16.) Cell adhesion molecules (CAMs) have been shown to stimulate axonal growth

through homophilic and/or heterophilic interactions with other molecules. In addition,

interactions between adhesion molecules and their receptors can potentiate the effects of

growth factors upon cell biochemistry via shared signaling pathways. (Ruoslahti, E.

15   (1997) Kidney Int. 51:1413-1417.) Cadherins comprise a family of calcium-dependant

glycoproteins that function in mediating cell-cell adhesion in solid tissues of multicellular

organisms. Integrins are ubiquitous transmembrane adhesion molecules that link cells to

the ECM by interacting with the cytoskeleton. Integrins also function as signal

transduction receptors and stimulate changes in intracellular calcium levels and protein

20   kinase activity. (Sjaastad, M.D. and Nelson, W.J. (1997) BioEssays 19:47-55.)   Lectins

are proteins characterized by their ability to bind carbohydrates on cell membranes by

means of discrete, modular carbohydrate recognition domains, CRDs. (Kishore, U. et al.

(1997) Matrix Biol. 15:583-592.) Certain cytokines and membrane-spanning proteins

have CRDs which may enhance interactions with extracellular or intracellular ligands,

25   with proteins in secretory pathways, or with molecules in signal transduction pathways.

The lipocalin superfamily constitutes a phylogenetically conserved group of more than

forty proteins that function by binding to and transporting a variety of physiologically

important ligands. Members of this family function as carriers of retinoids, odorants,

chromophores, pheromones, and sterols, and a subset of these proteins may be

30   multifunctional, serving as either a biosynthetic enzyme or as a specific enzyme inhibitor.

(Tanaka, T. et al. (1997) J. Biol. Chem. 272:15789-15795; and van't Hof, W. et al. (1997)

J. Biol. Chem. 272:1837-1841.) Selectins are a family of calcium ion-dependent lectins

expressed on inflamed vascular endothelium and the surface of some leukocytes. They

mediate rolling movement and adhesive contacts between blood cells and blood vessel

walls. The structure of the selectins and their ligands supports the type of bond formation

and dissociation that allows a cell to roll under conditions of flow. (Rossiter, H. et al.

5   (1997) Mol. Med. Today 3:214-222.)

Protein kinases regulate many different cell proliferation, differentiation, and

signaling processes by adding phosphate groups to proteins. Reversible protein

phosphorylation is a key strategy for controlling protein functional activity in eukaryotic

cells. The high energy phosphate which drives this activation is generally transferred from

10   adenosine triphosphate molecules (ATP) to a particular protein by protein kinases and

removed from that protein by protein phosphatases. Phosphorylation occurs in response to

extracellular signals, cell cycle checkpoints, and environmental or nutritional stresses.

Protein kinases may be roughly divided into two groups; protein tyrosine kinases (PTKs)

which phosphorylate tyrosine residues, and serine/threonine kinases (STKs) which

15   phosphorylate serine or threonine residues. A few protein kinases have dual specificity. A

majority of kinases contain a similar 250-300 amino acid catalytic domain which can be

further divided into eleven subdomains. The N-terminal domain, which contains

subdomains I to IV, generally folds into a two-lobed structure which binds and orients the

ATP (or GTP) donor molecule. The larger C terminal domain, which contains

20   subdomains VIA to XI, binds the protein substrate and carries out the transfer of the

gamma phosphate from ATP to the hydroxyl group of the target amino acid residue.

Subdomain V links the two domains. Each of the 11 subdomains contain specific residues

and motifs that are characteristic and are highly conserved. (Hardie, G. and Hanks, S.

(1995) The Protein Kinase Facts Book, Vol I, pp. 7-47, Academic Press, San Diego, CA.)

25

Protein phosphatases remove phosphate groups from molecules previously

modified by protein kinases thus participating in cell signaling, proliferation,

differentiation, contacts, and oncogenesis. Protein phosphorylation is a key strategy used

to control protein functional activity in eukaryotic cells. The high energy phosphate is

30   transferred from ATP to a protein by protein kinases and removed by protein

phosphatases. There appear to be three evolutionarily-distinct protein phosphatase gene

families: protein phosphatases (PPs); protein tyrosine phosphatases (PTPs); and

acid/alkaline phosphatases (APs). PPs dephosphorylate phosphoserine/threonine residues

and are an important regulator of many cAMP mediated, hormone responses in cells.

PTPs reverse the effects of protein tyrosine kinases and therefore play a significant role in

cell cycle and cell signaling processes. Although APs dephosphorylate substrates in vitro,

5   their role in vivo is not well known. (Carbonneau, H. and Tonks, N.K. (1992) Annu. Rev.

Cell Biol. 8:463-493.)

Protein phosphatase inhibitors control the activities of specific phosphatases. A

specific inhibitor of PP-I, I-1, has been identified that when phosphorylated by cAMP-

dependent protein kinase (PKA) specifically binds to PP-I and inhibits its activity. Since

10   PP-I is dephosphoryles many of the proteins phosphorylated by PKA, activation of I-1 by

PKA serves to amplify the effects of PKA and the many cAMP-dependent responses

mediated by PKA. In addition, since PP-I also dephosphorylates many phosphoproteins

that are not phosphorylated by PKA, I-1 activation serves to exert cAMP control over

other protein phosphorylations. I_1PP2A is a specific and potent inhibitor of PP-IIA. (Li,

15   M. et al. (1996) Biochemistry 35:6998-7002.) Since PP-IIA is the main phosphatase

responsible for reversing the phosphorylations of serine/threonine kinases, I_1PP2A has

broad effects in controlling protein phosphorylations.

Cyclic nucleotides (cAMP and cGMP) function as intracellular second messengers

to transduce a variety of extracellular signals, including hormones, and light and

20   neurotransmitters. Cyclic nucleotide phosphodiesterases (PDEs) degrade cyclic

nucleotides to their corresponding monophosphates, thereby regulating the intracellular

concentrations of cyclic nucleotides and their effects on signal transduction. At least

seven families of mammalian PDEs have been identified based on substrate specificity and

affinity, sensitivity to cofactors and sensitivity to inhibitory drugs. (Beavo, J.A. (1995)

25   Physiological Reviews 75: 725-748.) PDEs are composed of a catalytic domain of ~270

amino acids, an N-terminal regulatory domain responsible for binding cofactors and, in

some cases, a C-terminal domain with unknown function. Within the catalytic domain,

there is approximately 30% amino acid identity between PDE families and ~85-95%

identity between isozymes of the same family. Furthermore, within a family there is

30   extensive similarity (>60%) outside the catalytic domain, while across families there is

little or no sequence similarity. A variety of diseases have been attributed to increased

PDE activity and inhibitors of PDEs have been used effectively as anti-inflammatory.

antihypertensive, and antithrombotic agents. (Verghese, M.W. et al. (1995) Mol. Pharmacol. 47:1164-1171; and Banner, K.H.. and Page, C.P. (1995) Eur. Respir. J. 8:996-1000.)

5   Phospholipases (PLs) are enzymes that catalyze the removal of fatty acid residues from phosphoglycerides. PLs play an important role in transmembrane signal transduction and are named according to the specific ester bond in phosphoglycerides that is hydrolyzed, i.e., A_1, A_2, C or D. PLA_2 cleaves the ester bond at position 2 of the glycerol moiety of membrane phospholipids giving rise to arachidonic acid. Arachidonic acid is the common precursor to four major classes of eicosanoids; prostaglandins, prostacyclins,

10   thromboxanes and leukotrienes. Eicosanoids are signaling molecules involved in the contraction of smooth muscle, platelet aggregation, and pain and inflammatory responses. PLC is an important link in certain receptor-mediated, signaling transduction pathways. Extracellular signaling molecules including hormones, growth factors, neurotransmitters, and immunoglobulins bind to their respective cell surface receptors and activate PLC.

15   Activated PLC generates second messenger molecules from the hydrolysis of inositol phospholipids that regulate cellular processes, e.g., secretion, neural activity, metabolism and proliferation. (Alberts, B. et al. (1994) Molecular Biology of The Cell, Garland Publishing, Inc., New York, NY, pp. 85, 211, 239-240, 642-645.)

        The nucleotide cyclases, i.e., adenylate and guanylate cyclase, catalyze the

20   synthesis of the cyclic nucleotides, cAMP and cGMP, from ATP and GTP, respectively. They act in concert with phosphodiesterases, which degrade cAMP and cGMP, to regulate the cellular levels of these molecules and their functions. cAMP and cGMP function as intracellular second messengers to transduce a variety of extracellular signals, e.g., hormones, and light and neurotransmitters. Adenylate cyclase is a plasma membrane

25   protein that is coupled with various hormone receptors also located on the plasma membrane. Binding of a hormone to its receptor activates adenylate cyclase which, in turn, increases the levels of cAMP in the cytosol. The activation of other molecules by cAMP leads to the cellular effect of the hormone. In a similar manner, guanylate cyclase participates in the process of visual excitation and phototransduction in the eye. (Stryer,

30   L. (1988) Biochemistry W.H. Freeman and Co., New York, pp. 975-980, 1029-1035.) Cytokines are produced in response to cell perturbation. Some cytokines are produced as precursor forms, and some form multimers in order to become active. They are produced

in groups and in patterns characteristic of the particular stimulus or disease, and the members of the group interact with one another and other molecules to produce an overall biological response. Interleukins, neurotrophins, growth factors, interferons, and chemokines are all families of cytokines which work in conjunction with cellular receptors

5   to regulate cell proliferation and differentiation and to affect such activities, e.g., leukocyte migration and function, hematopoietic cell proliferation, temperature regulation, acute response to infections, tissue remodeling, and cell survival. Studies using antibodies or other drugs that modify the activity of a particular cytokine are used to elucidate the roles of individual cytokines in pathology and physiology.

10   Chemokines are a small chemoattractant cytokines which are active in leukocyte trafficking. Initially, chemokines were isolated and purified from inflamed tissues, but recently several chemokines have been discovered through molecular cloning techniques. Chemokines have been shown to be active in cell activation and migration, angiogenic and angiostatic activities, suppression of hematopoiesis, HIV infectivity, and promoting Th-

15   1(IL-2-, interferon γ-stimulated) cytokine release.

           Chemokines generally contain 70-100 amino acids and are subdivided into four subfamilies based on the presence and arrangement of conserved CXC, CC, CX3C and C motifs. The CXC (alpha), CC (beta), and CX3C chemokines contain four conserved cysteines. The CC subfamily is active on monocytes, lymphocytes, eosinophils, and mast

20   cells; the CXC subfamily, on neutrophils; CX3C and C subfamilies, on T-cells. Many of the CC chemokines have been characterized functionally as well as structurally. (Callard, R. and Gearing, A. (1994) The Cytokine Facts Book, Academic Press, New York, NY, pp. 181-190, 210-213, 223-227.)

           Growth and differentiation factors function in intercellular communication. Once

25   secreted from the cell, some factors require oligomerization or association with ECM in order to function. Complex interactions among these factors and their receptors result in the stimulation or inhibition of cell division, cell differentiation, cell signaling, and cell motility. Some factors act on their cell of origin (autocrine signaling); on neighboring cells (paracrine signaling); or on distant cells (endocrine signaling).

30   There are three broad classes of growth and differentiation factors. The first class includes the large polypeptide growth factors, e.g., epidermal growth factor, fibroblast growth factor, transforming growth factor, insulin-like growth factor, and platelet-derived

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growth factor. Each of these defines a family of related molecules which stimulate cell proliferation for wound healing, bone synthesis and remodeling, and regeneration of epithelial, epidermal, and connective tissues, and induce differentiation of embryonic tissues. Nerve growth factor functions specifically as a neurotrophic factor, and all induce

5   differentiation of embryonic tissues. The second class includes the hematopoietic growth factors which stimulate the proliferation and differentiation of blood cells such as B-lymphocytes, T-lymphocytes, erythrocytes, platelets, eosinophils, basophils, neutrophils, macrophages, and their stem cell precursors. These factors include colony-stimulating factors, erythropoietin, and cytokines, e.g., interleukins, interferons (IFNs), and tumor

10   necrosis factor (TNF). Cytokines are secreted by cells of the immune system and function in immunomodulation. The third class includes small peptide factors e.g., bombesin, vasopressin, oxytocin, endothelin, transferrin, angiotensin II, vasoactive intestinal peptide, and bradykinin, which function as hormones to regulate cellular functions other than proliferation.

15   Growth and differentiation factors have been shown to play critical roles in neoplastic transformation of cells in vitro and in tumor progression in vivo. Inappropriate expression of growth factors by tumor cells may contribute to vascularization and metastasis of melanotic tumors. In hematopoiesis, growth factor misregulation can result in anemias, leukemias and lymphomas. Certain growth factors, e.g., IFN, are cytotoxic to

20   tumor cells both in vivo and in vitro. Moreover, growth factors and/or their receptors are related both structurally and functionally related to oncoproteins. In addition, growth factors affect transcriptional regulation of both proto-oncogenes and oncosuppressor genes. (Pimentel, E. (1994) Handbook of Growth Factors, CRC Press, Ann Arbor, MI, pp. 6-25.)

25   Proteolytic enzymes or proteases degrade proteins by reducing the activation energy needed for the hydrolysis of peptide bonds. The major families are the zinc, serine, cysteine, thiol, and carboxyl proteases.

Zinc proteases, e.g., carboxypeptidase A, have a zinc ion bound to the active site, recognize C-terminal residues that contain an aromatic or bulky aliphatic side chain, and

30   hydrolyze the peptide bond adjacent to the C-terminal residues. Serine proteases have an active site serine residue and include digestive enzymes, e.g., trypsin and chymotrypsin, components of the complement and blood-clotting cascades, and enzymes that control the

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degradation and turnover of extracellular matrix (ECM) molecules. Subfamilies of serine proteases include tryptases (cleavage after arginine or lysine), aspases (cleavage after aspartate), chymases (cleavage after phenylalanine or leucine), metases (cleavage after methionine), and serases (cleavage after serine). Cysteine proteases (e.g. cathepsin) are

5 produced by monocytes, macrophages and other immune cells and are involved in diverse cellular processes ranging from the processing of precursor proteins to intracellular degradation. Overproduction of these enzymes can cause the tissue destruction associated with rheumatoid arthritis and asthma. Thiol proteases, e.g., papain, contain an active site cysteine and are widely distributed within tissues. Thiol proteases effect catalysis through

10 a thiol ester intermediate facilitated by a proximal histidine side chain. Carboxyl proteases, e.g., pepsin, are active only under acidic conditions (pH 2 to 3). The active site of pepsin contains two aspartate residues; when one aspartate is ionized and the other is not, the enzyme is active. A common feature of the carboxyl proteases is that they are inhibited by very low concentrations (10^{-10} M) of the inhibitor pepstatin. A substrate

15 analog which induces structural changes at the active site of a protease functions as an antagonist or inhibitor.

Guanosine triphosphate-binding proteins (G proteins) participate in intracellular signal transduction and control regulatory pathways through cell surface receptors. These receptors respond to hormones, growth factors, neuromodulators, or other signaling

20 molecules, by binding GTP. Binding of GTP leads to the production of cAMP which controls phosphorylation and activation of other proteins. During this process, the hydrolysis of GTP acts as an energy source as well as an on-off switch for the GTPase activity.

The G proteins are small proteins which consist of single 21-30 kDa polypeptides.

25 They can be classified into five subfamilies: Ras, Rho, Ran, Rab, and ADP-ribosylation factor. These proteins regulate cell growth, cell cycle control, protein secretion, and intracellular vesicle interaction. In particular, the Ras proteins are essential in transducing signals from receptor tyrosine kinases to serine/threonine kinases which control cell growth and differentiation. Mutant Ras proteins, which bind but can not hydrolyze GTP,

30 are permanently activated and cause continuous cell proliferation or cancer.

All five subfamilies share common structural features and four conserved motifs, I to IV. Motif I is the most variable and has the signature of GXXXXGK, in which lysine

interacts with the β- and γ-phosphate groups of GTP. Motif II, III, and IV have DTAGQE, NKXD, and EXSAX as their respective signatures and regulate the binding of g-phosphate, GTP, and the guanine base of GTP, respectively. Most of the membrane-bound G proteins require a carboxy terminal isoprenyl group (CAAX), added

5   posttranslationally, for membrane association and biological activity. The G proteins also have a variable effector region, located between motifs I and II, which is characterized as the interaction site for guanine nucleotide exchange factors or GTPase-activating proteins.

Eukaryotic cells are bound by a membrane and subdivided into membrane bound compartments. As membranes are impermeable to many ions and polar molecules,

10   transport of these molecules is mediated by ion channels, ion pumps, transport proteins, or pumps. Symporters and antiporters regulate cytosolic pH by transporting ions and small molecules, e.g., amino acids, glucose, and drugs, across membranes; symporters transport small molecules and ions in the same direction, and antiporters, in the opposite direction. Transporter superfamilies include facilitative transporters and active ATP binding cassette

15   transporters involved in multiple-drug resistance and the targeting of antigenic peptides to MHC Class I molecules. These transporters bind to a specific ion or other molecule and undergo conformational changes in order to transfer the ion or molecule across a membrane. Transport can occur by a passive, concentration-dependent mechanism or can be linked to an energy source such as ATP hydrolysis or an ion gradient.

20   Ion channels are formed by transmembrane proteins which form a lined passageway across the membrane through which water and ions, e.g., Na^+, K^+, Ca^{2-}, and Cl^-, enter and exit the cell. For example, chloride channels are involved in the regulation of the membrane electric potential as well as absorption and secretion of ions across the membrane. In intracellular membranes of the Golgi apparatus and endocytic vesicles,

25   chloride channels also regulate organelle pH. Electrophysiological and pharmacological studies suggest that a variety of chloride channels exist in different cell types and that many of these channels have one or more protein kinase phosphorylation sites.

Ion pumps are ATPases which actively maintain membrane gradients. Ion pumps can be grouped into three classes, e.g., P, V, and F, according to their structure and

30   function. All have one or more binding sites for ATP on the cytosolic face of the membrane. The P-class ion pumps consist of two α and two β transmembrane subunits, include Ca^{2+} ATPase and Na^+/K^- ATPase, and function in transporting H^+, Na^+, K^-, and

Ca^{2+} ions. The V- and F-class ion pumps have similar structures, a cytosolic domain

formed by at least five extrinsic polypeptides and at least 2 transmembrane proteins, and

only transport H^-. F class H^+ pumps have been identified from the membranes of

mitochondria and chloroplast, and V-class H^+ pumps regulate acidity inside lysosomes,

5   endosomes, and plant vacuoles.

A family of structurally related intrinsic membrane proteins known as facilitative

glucose transporters catalyze the movement of glucose and other selected sugars across the

plasma membrane. The proteins in this family contain a highly conserved, large

transmembrane domain made of 12 transmembrane α-helices, and several less conserved,

10   asymmetric, cytoplasmic and exoplasmic domains. (Pessin, J. E., and Bell, G.I. (1992)

Annu. Rev. Physiol. 54:911-930.)

Amino acid transport is mediated by Na^+ dependent amino acid transporters.

These transporters are involved in gastrointestinal and renal uptake of dietary and cellular

amino acids and the re-uptake of neurotransmitters. Transport of cationic amino acids is

15   mediated by the system y+ family members and the cationic amino acid transporter (CAT)

family. Members of the CAT family share a high degree of sequence homology, and each

contains 12-14 putative transmembrane domains. (Ito, K. and Groudine, M. (1997) J.

Biol. Chem. 272:26780-26786.)

Proton-coupled, 12 membrane-spanning domain transporters such as PEPT 1 and

20   PEPT 2 are responsible for gastrointestinal absorption and for renal reabsorbtion of

peptides using an electrochemical H^+ gradient as the driving force. A heterodimeric

peptide transporter, consisting of TAP 1 and TAP 2, is associated with antigen processing.

Peptide antigens are transported across the membrane of the endoplasmic reticulum so

they can be presented to the major histocompatibility complex class I molecules. Each

25   TAP protein consists of multiple hydrophobic membrane spanning segments and a highly

conserved ATP-binding cassette. (Boll, M. et al. (1996) Proc. Natl. Acad. Sci.

93:284-289.)

Hormones are secreted molecules that circulate in the body fluids and bind to

specific receptors on the surface of, or within, target tissue cells. Although they have

30   diverse biochemical compositions and mechanisms of action, hormones can be grouped

into two categories. One category consists of small lipophilic molecules that diffuse

through the plasma membrane of target cells, bind to cytosolic or nuclear receptors, and

form a complex alters gene expression. Examples of this category include retinoic acid, thyroxine, and the cholesterol derived steroid hormones, progesterone, estrogen, testosterone, cortisol, and aldosterone. These hormones have a long half-life, e.g., several hours to days, and long-term effects of their target cells. Their solubility in the blood may

5   be increased by their association with carrier molecules. Within the target cell nucleus, hormone/receptor complexes bind to specific response elements in target gene regulatory regions.

A second category consists of hydrophilic hormones that function by binding to cell surface receptors and transducing the signal across the plasma membrane. Examples

10 of this category include amino acid derivatives, such as catecholamines, e.g., epinephrine, norepinephrine, and histamine; peptide hormones, e.g., glucagon, insulin, gastrin, secretin, cholecystokinin, adrenocorticotropic hormone, follicle stimulating hormone, luteinizing hormone, thyroid stimulating hormone, parathormone, and vasopressin. Peptide hormones are synthesized as inactive forms and stored in secretory vesicles. These hormones are

15 activated by protease cleavage before being released from the cell. Many hydrophilic hormones have a very short half-life and effect, e.g., seconds to hours, and are inactivated by proteases in the blood. (Lodish et al. (1995) Molecular Cell Biology, Scientific American Books Inc., New York, NY, pp. 856-864.)

Neuropeptides and vasomediators (NP/VM) comprise a large family of endogenous

20 signaling molecules. Included in the family are neurotransmitters such as bombesin, neuropeptide Y, neurotensin, neuromedin N, melanocortins, opioids, e.g., enkephalins, endorphins and dynorphins, galanin, somatostatin, tachykinins, vasopressin, and vasoactive intestinal peptide, and circulatory system-borne signaling molecules, e.g., angiotensin, complement, calcitonin, endothelins, formyl-methionyl peptides, glucagon,

25 cholecystokinin and gastrin. These proteins are synthesized as "pre-pro" molecules, and are activated and inactivated by proteolytic cleavage. NP/VMs can transduce signals directly, modulate the activity or release of other neurotransmitters and hormones, and act as catalytic enzymes in cascades. The effects of NP/VMs range from extremely brief or long-lasting (melanocortin-mediated changes in skin melanin).   Regulatory molecules

30 turn individual genes or groups of genes on and off in response to various inductive mechanisms of the cell or organism; act as transcription factors by determining whether or not transcription is initiated, enhanced, or repressed; and splice transcripts as dictated in a

particular cell or tissue. Although they interact with short stretches of DNA scattered throughout the entire genome, most gene expression is regulated near the site at which transcription starts or within the open reading frame of the gene being expressed. The regulated stretches of the DNA can be simple and interact with only a single protein, or

5   they can require several proteins acting as part of a complex to regulate gene expression. The external features of the double helix which provide recognition sites are hydrogen bond donor and acceptor groups, hydrophobic patches, major and minor grooves, and regular, repeated stretches of sequences which cause distinct bends in the helix. The surface features of the regulatory molecule are complementary to those of the DNA.

10   Many of the transcription factors incorporate one of a set of DNA-binding structural motifs, each of which contains either α helices or ß sheets and binds to the major groove of DNA. Seven of the structural motifs common to transcription factors are helix-turn-helix, homeodomains, zinc finger, steroid receptor, ß sheets, leucine zipper, and helix-loop-helix. (Pabo, C.O. and R.T. Sauer (1992) Ann. Rev. Biochem. 61:1053-95.) Other

15   domains of transcription factors may form crucial contacts with the DNA. In addition, accessory proteins provide important interactions which may convert a particular protein complex to an activator or a repressor or may prevent binding. (Alberts, B. et al. (1994) Molecular Biology of the Cell, Garland Publishing Co, New York, NY pp. 401-474.)

            The discovery of new human signal peptide-containing proteins and the

20   polynucleotides encoding these molecules satisfies a need in the art by providing new compositions which are useful in the diagnosis, treatment, and prevention of cancer and immunological disorders.

SUMMARY OF THE INVENTION

25   The invention features a substantially purified human signal peptide-containing protein (SIGP), having an amino acid sequence selected from the group consisting of SEQ ID NO:1 SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15.

30   The invention further provides isolated and substantially purified polynucleotides encoding SIGP. In a particular aspect, the polynucleotide has a nucleic acid sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17. SEQ ID NO:18.

SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30.

In addition, the invention provides a polynucleotide, or fragment thereof, which

5 hybridizes to any of the polynucleotides encoding an SIGP selected from the group consisting of SEQ ID NO:1 SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15. In another aspect, the invention provides a composition comprising isolated and purified

10 polynucleotides selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, or a fragment thereof.

The invention further provides a polynucleotide comprising the complement, or

15 fragments thereof, of any one of the polynucleotides encoding SIGP. In another aspect, the invention provides compositions comprising isolated and purified polynucleotides comprising the complement of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and

20 SEQ ID NO:30, or fragments thereof.

The present invention further provides an expression vector containing at least a fragment of any one of the polynucleotides selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ

25 ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30. In yet another aspect, the expression vector containing the polynucleotide is contained within a host cell.

The invention also provides a method for producing a polypeptide or a fragment thereof, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide encoding SIGP under

30 conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a

substantially purified SIGP in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to SIGP, as well as a purified agonist and a purified antagonist of SIGP.

The invention also provides a method for treating or preventing a cancer associated

5 with the decreased expression or activity of SIGP, the method comprising the step of administering to a subject in need of such treatment an effective amount of a pharmaceutical composition containing SIGP.

The invention also provides a method for treating or preventing a cancer associated with the increased expression or activity of SIGP, the method comprising the step of

10 administering to a subject in need of such treatment an effective amount of an antagonist of SIGP.

The invention also provides a method for treating or preventing an immune response associated with the increased expression or activity of SIGP, the method comprising the step of administering to a subject in need of such treatment an effective

15 amount of an antagonist of SIGP.

The invention also provides a method for detecting a nucleic acid sequence which encodes a human regulatory proteins in a biological sample, the method comprising the steps of: a) hybridizing a nucleic acid sequence of the biological sample to a polynucleotide sequence complementary to the polynucleotide encoding SIGP, thereby

20 forming a hybridization complex; and b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of the nucleic acid sequence encoding the human regulatory protein in the biological sample.

The invention also provides a microarray containing at least a fragment of at least one of the polynucleotides encoding a polypeptide having an amino acid sequence selected

25 from the group consisting of SEQ ID NO:1 SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15.

The invention also provides a method for detecting the expression level of a

30 nucleic acid encoding a human regulatory protein in a biological sample, the method comprising the steps of hybridizing the nucleic acid sequence of the biological sample to a complementary polynucleotide, thereby forming hybridization complex; and determining

expression of the nucleic acid sequence encoding a human regulatory protein in the biological sample by identifying the presence of the hybridization complex. In a preferred embodiment, prior to the hybridizing step, the nucleic acid sequences of the biological sample are amplified and labeled by the polymerase chain reaction.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"SIGP," as used herein, refers to the amino acid sequences of substantially purified SIGP obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist," as used herein, refers to a molecule which, when bound to SIGP, increases or prolongs the duration of the effect of SIGP. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of SIGP.

5   An "allele" or an "allelic sequence," as these terms are used herein, is an alternative form of the gene encoding SIGP. Alleles may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise

10   to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding SIGP, as described herein, include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a

15   polynucleotide the same SIGP or a polypeptide with at least one functional characteristic of SIGP. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding SIGP, and improper or unexpected hybridization to alleles, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding SIGP. The encoded

20   protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent SIGP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of SIGP is retained.

25   For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

30   The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments", "immunogenic

fragments", or "antigenic fragments" refer to fragments of SIGP which are preferably about 5 to about 15 amino acids in length and which retain some biological activity or immunological activity of SIGP. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid

5   sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification," as used herein, relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art. (See, e.g., Dieffenbach, C.W. and

10   G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, pp.1-5.)

The term "antagonist," as it is used herein, refers to a molecule which, when bound to SIGP, decreases the amount or the duration of the effect of the biological or immunological activity of SIGP. Antagonists may include proteins, nucleic acids,

15   carbohydrates, antibodies, or any other molecules which decrease the effect of SIGP.

As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fa, F(ab')$_2$, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind SIGP polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing

20   antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

25   The term "antigenic determinant," as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic

30   determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense," as used herein, refers to any composition containing a

nucleic acid sequence which is complementary to a specific nucleic acid sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides

5   combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

As used herein, the term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise,

10   "immunologically active" refers to the capability of the natural, recombinant, or synthetic SIGP, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by

15   base pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and

20   strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition comprising a given amino acid sequence," as these terms are used herein, refer broadly to

25   any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation, an aqueous solution, or a sterile composition. Compositions comprising polynucleotides encoding SIGP, e.g., SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ

30   ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID 30, or fragments thereof, may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the

probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents
(e.g., SDS) and other components (e.g., Denhardt's solution, dry milk, salmon sperm
DNA, etc.).

The phrase "consensus sequence," as used herein, refers to a nucleic acid sequence
5   which has been resequenced to resolve uncalled bases, extended using XL-PCR™ (Perkin
Elmer, Norwalk, CT) in the 5' and/or the 3' direction, and resequenced, or which has been
assembled from the overlapping sequences of more than one Incyte Clone using a
computer program for fragment assembly, such as the GELVIEW™ Fragment Assembly
system (GCG, Madison, WI). Some sequences have been both extended and assembled to
10   produce the consensus sequence .

As used herein, the term "correlates with expression of a polynucleotide" indicates
that the detection of the presence of nucleic acids, the same or related to a nucleic acid
sequence encoding SIGP, by northern analysis is indicative of the presence of nucleic
acids encoding SIGP in a sample, and thereby correlates with expression of the transcript
15   from the polynucleotide encoding SIGP.

The term "SIGP" refers to any or all of the human polypeptides, SIGP-1, SIGP-2,
SIGP-3, SIGP-4, SIGP-5, SIGP-6, SIGP-7, SIGP-8, SIGP-9, SIGP-10, SIGP-11, SIGP-12,
SIGP-13, SIGP-14, and SIGP-15.

A "deletion," as the term is used herein, refers to a change in the amino acid or
20   nucleotide sequence that results in the absence of one or more amino acid residues or
nucleotides.

The term "derivative," as used herein, refers to the chemical modification of SIGP,
of a polynucleotide sequence encoding SIGP, or of a polynucleotide sequence
complementary to a polynucleotide sequence encoding SIGP. Chemical modifications of a
25   polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl,
acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at
least one biological or immunological function of the natural molecule. A derivative
polypeptide is one modified by glycosylation, pegylation, or any similar process that
retains at least one biological or immunological function of the polypeptide from which it
30   was derived.

The term "homology," as used herein, refers to a degree of complementarity.
There may be partial homology or complete homology. The word "identity" may

substitute for the word "homology." A partially complementary sequence that at least

partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred

to as "substantially homologous." The inhibition of hybridization of the completely

complementary sequence to the target sequence may be examined using a hybridization

5   assay (Southern or northern blot, solution hybridization, and the like) under conditions of

reduced stringency. A substantially homologous sequence or hybridization probe will

compete for and inhibit the binding of a completely homologous sequence to the target

sequence under conditions of reduced stringency. This is not to say that conditions of

reduced stringency are such that non-specific binding is permitted, as reduced stringency

10   conditions require that the binding of two sequences to one another be a specific (i.e., a

selective) interaction. The absence of non-specific binding may be tested by the use of a

second target sequence which lacks even a partial degree of complementarity (e.g., less

than about 30% homology or identity). In the absence of non-specific binding, the

substantially homologous sequence or probe will not hybridize to the second non-

15   complementary target sequence.

The phrases "percent identity" or "% identity" refer to the percentage of sequence

similarity found in a comparison of two or more amino acid or nucleic acid sequences.

Percent identity can be determined electronically, e.g., by using the MegAlign program

(Lasergene software package, DNASTAR, Inc., Madison WI). The MegAlign program

20   can create alignments between two or more sequences according to different methods, e.g.,

the Clustal Method. (Higgins, D.G. and Sharp, P.M. (1988) Gene 73:237-244.) The

Clustal algorithm groups sequences into clusters by examining the distances between all

pairs. The clusters are aligned pairwise and then in groups. The percentage similarity

between two amino acid sequences, e.g., sequence A and sequence B, is calculated by

25   dividing the length of sequence A, minus the number of gap residues in sequence A, minus

the number of gap residues in sequence B, into the sum of the residue matches between

sequence A and sequence B, times one hundred. Gaps of low or of no homology between

the two amino acid sequences are not included in determining percentage similarity.

Percent identity between nucleic acid sequences can also be calculated by the Clustal

30   Method, or by other methods known in the art, such as the Jotun Hein Method. (See, e.g.,

Hein, J. (1990) Methods in Enzymology 183:626-645.) Identity between sequences can

also be determined by other methods known in the art, e.g., by varying hybridization

conditions.

"Human artificial chromosomes" (HACs), as described herein, are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance. (See, e.g., Harrington, J.J. et al. (1997) Nat Genet. 15:345-355.)

The term "humanized antibody," as used herein, refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization," as the term is used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

As used herein, the term "hybridization complex" as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" or "addition," as used herein, refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term "microarray," as used herein, refers to an array of distinct polynucleotides or oligonucleotides arrayed on a substrate, such as paper, nylon or any other type of membrane, filter, chip, glass slide, or any other suitable solid support.

The term "modulate," as it appears herein, refers to a change in the activity of SIGP. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of SIGP.

The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to an oligonucleotide, nucleotide, polynucleotide, or any fragment thereof, to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-

5   like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which are greater than about 60 nucleotides in length, and most preferably are at least about 100 nucleotides, at least about 1000 nucleotides, or at least about 10,000 nucleotides in length.

The terms "operably associated" or "operably linked," as used herein, refer to

10   functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the transcription of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the encoded polypeptide but still bind to operator sequences that

15   control expression of the polypeptide.

The term "oligonucleotide," as used herein, refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. As used herein, the term "oligonucleotide" is

20   substantially equivalent to the terms "amplimers," "primers," "oligomers," and "probes," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA), as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal

25   lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA and RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell. (See, e.g., Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63.)

The term "sample," as used herein, is used in its broadest sense. A biological

30   sample suspected of containing nucleic acids encoding SIGP, or fragments thereof, or SIGP itself may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or

bound to a solid support; a tissue; a tissue print; etc.

As used herein, the terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein

5   recognized by the binding molecule (i.e., the antigenic determinant or epitope). For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

As used herein, the term "stringent conditions" refers to conditions which permit

10   hybridization between polynucleotide sequences and the claimed polynucleotide sequences. Suitably stringent conditions can be defined by, for example, the concentrations of salt or formamide in the prehybridization and hybridization solutions, or by the hybridization temperature, and are well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of

15   formamide, or raising the hybridization temperature.

For example, hybridization under high stringency conditions could occur in about 50% formamide at about 37°C to 42°C. Hybridization could occur under reduced stringency conditions in about 35% to 25% formamide at about 30°C to 35°C. In particular, hybridization could occur under high stringency conditions at 42°C in 50%

20   formamide, 5X SSPE, 0.3% SDS, and 200 μg/ml sheared and denatured salmon sperm DNA. Hybridization could occur under reduced stringency conditions as described above, but in 35% formamide at a reduced temperature of 35°C. The temperature range corresponding to a particular level of stringency can be further narrowed by calculating the purine to pyrimidine ratio of the nucleic acid of interest and adjusting the temperature

25   accordingly. Variations on the above ranges and conditions are well known in the art.

The term "substantially purified," as used herein, refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

30   A "substitution," as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Transformation," as defined herein, describes a process by which exogenous DNA

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enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host

5   cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, and refers to cells which transiently express the inserted DNA or RNA for

10   limited periods of time.

A "variant" of SIGP, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have

15   "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

20

THE INVENTION

The invention is based on the discovery of new human signal peptide-containing proteins, collectively referred to as SIGP and individually as SIGP-1, SIGP-2, SIGP-3, SIGP-4, SIGP-5, SIGP-6, SIGP-7, SIGP-8, SIGP-9, SIGP-10, SIGP-11, SIGP-12,

25   SIGP-13, SIGP-14, and SIGP-15, the polynucleotides encoding SIGP (SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30); and the use of these compositions for the diagnosis, treatment, or prevention of cancer and immunological

30   disorders. Table 1 shows the sequence identification numbers, Incyte Clone identification number, cDNA library, NCBI sequence identifier and GenBank species description for each of the human signal peptide-containing proteins disclosed herein.

TABLE 1

PROTEIN	NUCLEOTIDE	CLONE ID	LIBRARY	NCBI SEQ ID	HOMOLOG
SEQ ID NO:1	SEQ ID NO:16	866885	BRAITUT03	GI 1488683	Rattus norvegicus
SEQ ID NO:2	SEQ ID NO:17	1273453	TESTIUT02		
SEQ ID NO:3	SEQ ID NO:18	1534876	SPLNNOT04		
SEQ ID NO:4	SEQ ID NO:19	1634813	COLNNOT19	GI 219624	Mus musculus
SEQ ID NO:5	SEQ ID NO:20	1711840	PROSNOT16	GI 182651	Homo sapiens
SEQ ID NO:6	SEQ ID NO:21	1747327	STOMTUT02	GI 2062391	Homo sapien
SEQ ID NO:7	SEQ ID NO:22	1864292	PROSNOT19	GI 295671	Saccharomyces cerevisiae
SEQ ID NO:8	SEQ ID NO:23	1866437	THP1NOT01		
SEQ ID NO:9	SEQ ID NO:24	1871375	SKINBIT01		
SEQ ID NO:10	SEQ ID NO:25	1880830	LEUKNOT03	GI 1872521	Arabidopsis thaliana
SEQ ID NO:11	SEQ ID NO:26	2328134	COLNNOT11	GI 1911776	Homo sapiens
SEQ ID NO:12	SEQ ID NO:27	2652271	THYMNOT04	GI 895855	Solanum lycopersicum
SEQ ID NO:13	SEQ ID NO:28	2965248	SCORNOT04	GI 1478507	Homo sapiens
SEQ ID NO:14	SEQ ID NO:29	3057669	PONSAZT01	GI 260241	
SEQ ID NO:15	SEQ ID NO:30	3125156	LNODNOT05	GI 804750	Homo sapiens

Nucleic acids encoding the SIGP-1 of the present invention were first identified in Incyte Clone 866885 from the brain tumor cDNA library (BRAITUT03) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:16, was derived from Incyte Clones 866885 (BRAITUT03), 2991983 (KIDNFET02), 067954

5   (HUVESTB01), and 1499109 (SINTBST01).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1. SIGP-1 is 236 amino acids in length and has a potential N-glycosylation site at N199; two potential casein kinase II phosphorylation sites at S8 and T72; a potential N-myristoylation site at G169; and three potential protein

10   kinase C phosphorylation sites at T43, S96, and T201. SIGP-1 shares 24% identity with rat syntaxin (GI 1488683). The fragment of SEQ ID NO:16 from about nucleotide 43 to about nucleotide 93 is useful for hybridization. Northern analysis shows the expression of this sequence in hematopoietic and immune, reproductive, gastrointestinal, neural, cardiovascular, and developmental cDNA libraries. Approximately 43% of these libraries

15   are associated with neoplastic disorders, 26% with inflammation, and 19% with cell proliferation.

Nucleic acids encoding the SIGP-2 of the present invention were first identified in Incyte Clone 1273453 from the testicle cDNA library (TESTTUT02) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID

20   NO:17, was derived from Incyte Clones 1273453 (TESTTUT02), 1970337 (UCMCL5T01), 1218926 (NEUTGMT01), 1881349 (LEUKNOT03), and 1722377 (BLADNT06).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:2. SIGP-17 is 267 amino acids in length and has a

25   potential N glycosylation site at N230, five potential casein kinase II phosphorylation sites at S9, T45, T77, S190, and T263, and two potential protein kinase C phosphorylation sites at S232 and S236. The fragment of SEQ ID NO:17 from about nucleotide 140 to about nucleotide 175 is useful for hybridization. Northern analysis shows the expression of this sequence in reproductive, cardiovascular, and

30   hematopoietic and immune cDNA libraries. Approximately 42% of these libraries are associated with neoplastic disorders and 40% with immune response.

Nucleic acids encoding the SIGP-3 of the present invention were first identified in

Incyte Clone 1534876 from the spleen cDNA library (SPLNNOT04) using a computer

search for amino acid alignments. A consensus sequence, SEQ ID NO:18, was derived

from Incyte Clones 1253004 (LUNGFET03), 1382838 (BRAITUT08), 1532501

(SPLNNOT04), 1534876 (SPLNNOT04), 1705806 (DUODNOT02), 1738301

5   (COLNNOT22), 1926209 (BRSTNOT02), and shotgun sequences SAOA00587,

SAOA02048, and SAOA03535.

        In one embodiment, the invention encompasses a polypeptide comprising the

amino acid sequence of SEQ ID NO:3. SIGP-3 is 161 amino acids in length and has a

potential signal peptide sequence between M1 and C13. SIGP-3 also has 17 cysteine

10   residues with the potential for forming intramolecular disulfide bridges. Six of these

cysteine residues, between residues C129 and C152, are found in a signature sequence for

trypsin/alpha-amylase inhibitors that form a structure with intramolecular disulfide

bridges. SIGP-3 has two potential casein kinase II phosphorylation sites at T25 and S35;

and two potential protein kinase C phosphorylation sites at S35 and T87. The fragment of

15   SEQ ID NO:18 from about nucleotide 406 to about nucleotide 477, which encompasses

the trypsin/alpha-amylase inhibitor signature sequence, is useful for hybridization.

Northern analysis shows the expression of this sequence in gastrointestinal and male and

female reproductive cDNA libraries. Approximately 45% of these libraries are associated

with neoplastic disorders and 28% with inflammation and the immune response..

20   Nucleic acids encoding the SIGP-4 of the present invention were first identified in

Incyte Clone 1634813 from the cecal tissue cDNA library (COLNNOT19) using a

computer search for amino acid sequence alignments. A consensus sequence, SEQ ID

NO:19, was derived from Incyte Clones 1634813 (COLNNOT19), 2904583

(THYMNOT05), 1634813 (COLNNOT19), and 1310492 (COLNFET02), and shotgun

25   sequence SAPA04436.

        In one embodiment, the invention encompasses a polypeptide comprising the

amino acid sequence of SEQ ID NO:4. SIGP-4 is 150 amino acids in length and has one

potential N-glycosylation site at N139; and five potential phosphorylation sites at T48,

S118, S126, S135, and S136. SIGP-4 also has a potential signal peptide sequence

30   encompassing residues M1-A23. SIGP-4 shares 28% identity with mouse beta

chemokine, Exodus-2 (GI 2196924). The fragment of SEQ ID NO:19 from about

nucleotide 175 to about nucleotide 235 is useful for hybridization. Northern analysis

shows the expression of this sequence in gastrointestinal, developmental, hematopoietic, and immunological cDNA libraries. Approximately 50% of these libraries are associated with fetal development/cell proliferation and 25% with immune response.

5   Nucleic acids encoding the SIGP-5 of the present invention were first identified in Incyte Clone 1711840 from the prostate cDNA library (PROSNOT16) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:20, was derived from Incyte Clones 1711840 (PROSNOT16) and 2550483 (LUNGTUT06) and shotgun sequence SAQA03185.

       In one embodiment, the invention encompasses a polypeptide comprising the
10   amino acid sequence of SEQ ID NO:5. SIGP-5 is 118 amino acids in length and has three potential protein kinase C phosphorylation sites at S48, T103, and S109; and a potential signal peptide sequence from M1 to A20. SIGP-5 shares 61% identity with human midkine, a retinoic acid-responsive heparin binding factor involved in regulation of growth and differentiation (GI 182651). The fragment of SEQ ID NO:20 from about
15   nucleotide 511 to about nucleotide 555 is useful for hybridization. Northern analysis shows the expression of this sequence in reproductive, gastrointestinal, developmental, neural, and cardiovascular cDNA libraries. Approximately 58% of these libraries are associated with cancer, 16% with immune response, and 23% with fetal/proliferating cells.

20   Nucleic acids encoding the SIGP-6 of the present invention were first identified in Incyte Clone 1747327 from the stomach tumor cDNA library (STOMTUT02) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:21, was derived from Incyte Clones 475228 (MMLR2DT01), 1500771 (SINTBST01), 1880656 (LEUKNOT03), 1747327 (STOMTUT02), and 2720285
25   (LUNGTUT10).

       In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:6. SIGP-6 is 248 amino acids in length and has one potential N-glycosylation site at N56; three potential casein kinase II phosphorylation sites at S46, S134, and S140; and one potential protein kinase C
30   phosphorylation site at T217. SIGP-6 shares 100% identity with human K12 protein precursor which is expressed in breast cancer cells and peripheral blood leukocytes (GI 2062391). Northern analysis shows the expression of this sequence in gastrointestinal,

reproductive, hematopoietic/immune, and cardiovascular cDNA libraries.
Approximately 59% of these libraries are associated with cancer and 35% with immune response.

Nucleic acids encoding the SIGP-7 of the present invention were first identified
5   in Incyte Clone 1864292 from the diseased prostate cDNA library (PROSNOT19) using
a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID
NO:22, was derived from Incyte Clone 1864292 (PROSNOT19) and shotgun sequences
SARA02195, SARA03070, SARA03675, and SATA02454.

In one embodiment, the invention encompasses a polypeptide comprising the
10   amino acid sequence of SEQ ID NO:7. SIGP-7 is 404 amino acids in length and has
one potential amidation site at V136; one potential cAMP- and cGMP-dependent protein
kinase phosphorylation site at S66; twenty potential casein kinase II phosphorylation sites
at S23, T27, T74, S110, S111, S118, T122, S143, S145, S205, S207, S218, S219, S220,
T252, S254, S328, S330, S385, and T393; and twelve potential protein kinase C
15   phosphorylation sites at T27, S76, T81, S140, S161, S176, S229, T285, S309, S356, S367,
and S398. SIGP-7 shares 18% identity with the S. cerevisiae protein encoded by
SRP40, a weak suppressor of a mutant of the subunit AC40 of DNA-dependent RNA
polymerases I and II (GI 295671). The fragment of SEQ ID NO:22 f rom about
nucleotide 193 to about nucleotide 222 is useful for hybridization. Northern analysis
20   shows the expression of this sequence in reproductive, cardiovascular, and
hematopoietic/immune cDNA libraries. Approximately 75% of these libraries are
associated with cancer and 25% with immune response.

Nucleic acids encoding the SIGP-8 of the present invention were first identified
in Incyte Clone 1866437 from the human promonocyte cell line cDNA library
25   (THP1NOT01) using a computer search for amino acid sequence alignments. A
consensus sequence, SEQ ID NO:23, was derived from Incyte Clones 817970
(OVARTUT01), 825684 (PROSNOT06), 1866437 (THP1NOT01), 2190170
(PROSNOT26), and 3137972 (SMCCNOT02).

In one embodiment, the invention encompasses a polypeptide comprising the
30   amino acid sequence of SEQ ID NO:8. SIGP-8 is 405 amino acids in length and has
one potential N-glycosylation site at N378; one potential cAMP- and cGMP-

phosphorylation site at S332; nine potential casein kinase II phosphorylation sites at T34, S51, T77, S107, S158, S264, T266, S296, and S332; and one potential protein kinase C phosphorylation site at S68. The fragment of SEQ ID NO:23 from about nucleotide 85 to about nucleotide 144 is useful for hybridization. Northern analysis

5   shows the expression of this sequence in reproductive, hematopoietic/immune, neural, and developmental cDNA libraries. Approximately 37% of these libraries are associated with cancer, 33% with immune response, and 22% with fetal/proliferating cells.

Nucleic acids encoding the SIGP-9 of the present invention were first identified

10   in Incyte Clone 1871375 from the leg skin erythema nodosum cDNA library (SKINBIT01) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:24, was derived from Incyte Clones 1428052 (SINTBST01), 1871375 (SKINBIT01), and 3210563 (BLADNOT08).

In one embodiment, the invention encompasses a polypeptide comprising the

15   amino acid sequence of SEQ ID NO:9. SIGP-9 is 177 amino acids in length and has one potential casein kinase II phosphorylation site at S133; one potential glycosaminoglycan attachment site at S28GGG; and four potential protein kinase C phosphorylation sites at S44, S82, S115, and T148. SIGP-9 contains a signature sequence shared by the binding domains of receptors for lymphokines, hematopoietic

20   growth factors and growth hormone-related molecules at S52RWSLWS. The fragment of SEQ ID NO:24 encoding the sequence surrounding the receptor binding domain signature from about nucleotide 190 to about nucleotide 249 is useful for hybridization. Northern analysis shows the expression of this sequence in reproductive, cardiovascular, gastrointestinal, and developmental cDNA libraries. Approximately 44% of these

25   libraries are associated with cancer and 19% with immune response.

Nucleic acids encoding the SIGP-10 of the present invention were first identified in Incyte Clone 1880830 from the leukocyte cDNA library (LEUKNOT03) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:25, was derived from Incyte Clones 361577 (PROSNOT01); 2113591

30   (BRAITUT03); 1880830 (LEUKNOT03) and shotgun sequences SATA03292 and SATA00377.

In one embodiment, the invention encompasses a polypeptide comprising the

amino acid sequence of SEQ ID NO:10. SIGP-10 is 197 amino acids in length and has a potential cAMP- and cGMP-dependent protein kinase phosphorylation site at S121; and four potential protein kinase C phosphorylation sites at T3, S57, T107, and T153. SIGP-40 shares 15% identity with the <u>Arabidopsis thaliana</u> zinc-finger protein Lsd1 (GI

5   1872521). The fragment of SEQ ID NO:25 from about nucleotide 567 to about nucleotide 621 is useful for hybridization. Northern analysis shows the expression of this sequence in neural and reproductive cDNA libraries. Approximately 49% of these libraries are associated with neoplastic disorders, 24% with immune response, and 16% with fetal development.

10   Nucleic acids encoding the SIGP-11 of the present invention were first identified in Incyte Clone 2328134 from the colon cDNA library (COLNNOT11) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:26, was derived from Incyte Clones 2328134 (COLNNOT11), 1870180 (SKINBIT01), 081403 (SYNORAB01), and 851547 (NGANNOT01).

15   In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:11. SIGP-11 is 346 amino acids in length and has two potential cAMP- and cGMP-dependent protein kinase phosphorylation sites at residues S43 and S217; one potential casein kinase II phosphorylation site at residue T96; and five potential protein kinase C phosphorylation sites at residues T2, T15, T39,

20   T247, and S301. SIGP-50 shares 33% identity with the human putative rab5-interacting protein (GI 1911776) and the casein kinase II phosphorylation site at residue T96. The fragment of SEQ ID NO:26 encoding the potential extracellular ligand binding domain from about nucleotide 16 to about nucleotide 76 is useful for hybridization. Northern analysis shows the expression of this sequence in reproductive, gastrointestinal,

25   cardiovascular, and neural cDNA libraries. Approximately 44% of these libraries are associated with cancer, 28% are associated with immune response, and 20% with fetal disorders.

       Nucleic acids encoding the SIGP-12 of the present invention were first identified in Incyte Clone 2652271 from the thymus cDNA library (THYMNOT04) using a computer

30   search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:27, was derived from Incyte Clones 2652271 (THYMNOT04), 2742813 (BRSTTUT14), 763431

(BRAITUT02), 1272403 (TESTTUT02), 1240531 (LUNGNOT03), and 1318448 (BLADNOT04).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:12. SIGP-12 is 256 amino acids in length and has

5 three potential N glycosylation sites at N76, N106, and N212; three potential casein kinase II phosphorylation sites at T46, S188, and T204; two potential protein kinase C phosphorylation sites at S130 and S221; two potential ribonuclease T2 family histidine active sites from W62 to P69 and from F110 to C121; and a potential signal peptide sequence from M1 to A24. SIGP-59 shares 24% identity with Solanum lycopersicum

10 ribonuclease LE (GI 895855); 80% identity between W62 and P75, one of the two ribonuclease T2 family histidine active sites; and 92% identity between F110 and C121, the second of the two ribonuclease T2 family histidine active sites. The fragment of SEQ ID NO:27 from about nucleotide 462 to about nucleotide 494 is useful for hybridization. Northern analysis shows the expression of this sequence in reproductive, hematopoietic,

15 and gastrointestinal cDNA libraries. Approximately 53% of these libraries are associated with neoplastic disorders and 28% with immune response.

Nucleic acids encoding the SIGP-13 of the present invention were first identified in Incyte Clone 2965248 from the cervical spinal cord cDNA library (SCORNOT04) using a computer search for amino acid sequence alignments. A consensus sequence,

20 SEQ ID NO:28, was derived from Incyte Clones 2965248 (SCORNOT04), 485746 (HNT2RAT01), 865684 (BRAITUT03), 1459157 (COLNFET02), 1597772 (BRAINOT14), 531430 (BRAINOT03), 725362 (SYNOOAT01), 1620429 (BRAITUT13), and 190305 (SYNORAB01).

In one embodiment, the invention encompasses a polypeptide comprising the

25 amino acid sequence of SEQ ID NO:13. SIGP-13 is 235 amino acids in length and has seven potential cAMP- and cGMP-dependent protein kinase phosphorylation sites at S50, T80, T98, T126, S135, S136, and T194; three potential casein kinase II phosphorylation sites at S60, T80, and S81; six potential protein kinase C phosphorylation sites at S114, T119, T137, S142, S146, and S174; and a strathmin 1

30 family signature from P75 to E84. SIGP-28 shares 44% identity with human strathmin homolog SCG10/neuron-specific growth-associated protein in Alzheimer's disease (GI 1478503), and 71% identity between M1 and A107. In addition, one potential cAMP-

and cGMP-dependent protein kinase phosphorylation site, one potential casein kinase II phosphorylation site, the strathmin 1 family signature, and the hydrophobic transmembrane domains are conserved between these molecules. TM1 extends from about L15 to about F25; and TM2, from about G196 to about P212. The fragments of

5   SEQ ID NO:28 from about nucleotide 158 to about nucleotide 196 and from about nucleotide 614 to about nucleotide 643 are useful for hybridization. Northern analysis shows the expression of this sequence in neural, reproductive, gastrointestinal, and hematopoietic/immune cDNA libraries. Approximately 50% of these libraries are associated with neoplastic disorders and 19% with immune response.

10   Nucleic acids encoding the SIGP-14 of the present invention were first identified in Incyte Clone 3057669 from the pons cDNA library (PONSAZT01) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:29, was derived from Incyte Clones 3057669 (PONSAZT01), 548211 (BEPINOT01), 3702516 (PENCNOT07), 3581270 (293TF3T01), 495191 (HNT2NOT01), 2784427

15   (BRSTNOT13), 1515961 (PANCTUT01), 3552333 (SYNONOT01), 2838668 (DRGLNOT01), 14600680 (COLNFET02), and 285677 (EOSIHET02).

       In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:14. SIGP-14 is 371 amino acids in length and has three potential N-glycosylation sites at N70, N125, and N362; eleven potential casein

20   kinase II phosphorylation sites at T22, S66, S72, S73, S102, T160, T201, T215, T278, T285, and S316; seven potential protein kinase C phosphorylation sites at S72, T79, S99, T127, S134, S257, and T299; and one protein kinase signature and profile from L188 to F200. Northern analysis shows the expression of this sequence in gastrointestinal, reproductive, and neural cDNA libraries. Approximately 54% of these

25   libraries are associated with neoplastic disorders and 14% with immune response.

       Nucleic acids encoding the SIGP-15 of the present invention were first identified in Incyte Clone 3125156 from the lymph node cDNA library (LNODNOT05) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:30, was derived from Incyte Clones 3125156 (LNODNOT05), 1417459

30   (BRAINOT12), 1567861 (UTRSNOT05), 154233 (THP1PLB02), 872652 (LUNGAST01), 2525803 (BRAITUT21), and 1209172 (BRSTNOT02).

       In one embodiment, the invention encompasses a polypeptide comprising the

amino acid sequence of SEQ ID NO:15. SIGP-15 is 523 amino acids in length and has

one potential N glycosylation sites at N186; nine potential casein kinase II

phosphorylation sites at S63, T85, S179, S188, T210, S231, T269, T295, and S474; one

potential glycosaminoglycan attachment site at S335; ten potential protein kinase C

5   phosphorylation sites at T9, S159, S172, S179, T246, S263, S283, S416, S447, and S498;

two potential tyrosine kinase phosphorylation sites at Y106 and Y170; and one tyrosine

specific protein phosphatase active site at V331. SIGP-30 shares 21% identity with human

T-cell protein tyrosine phosphatase (GI 804750), the N186 glycosylation site, the

phosphorylation sites at S179, S188, T210, T246, S263, T295, S416, and Y170; and 50%

10   identity between P324 and F344, the region of the tyrosine specific protein phosphatase

active site. The fragments of SEQ ID NO:30 from about nucleotide 64 to about nucleotide

183 and from about nucleotide 1087 to about nucleotide 1119 are useful for hybridization.

Northern analysis shows the expression of this sequence in neural, reproductive, and

gastrointestinal cDNA libraries. Approximately 55% of these libraries are associated with

15   neoplastic disorders and 22% with immune response.

The invention also encompasses SIGP variants. A preferred SIGP variant is one

which has at least about 80%, more preferably at least about 90%, and most preferably at

least about 95% amino acid sequence identity to the SIGP amino acid sequence, and which

contains at least one functional or structural characteristic of SIGP.

20   The invention also encompasses polynucleotides which encode SIGP. Accordingly,

any nucleic acid sequence which encodes the amino acid sequence of SIGP can be used

to produce recombinant molecules which express SIGP. In a particular embodiment, the

invention encompasses a polynucleotide consisting of a nucleic acid sequence selected

from the group consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID

25   NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24,

SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and

SEQ ID NO:30.

It will be appreciated by those skilled in the art that as a result of the degeneracy of

the genetic code, a multitude of polynucleotide sequences encoding SIGP, some bearing

30   minimal homology to the polynucleotide sequences of any known and naturally occurring

gene, may be produced. Thus, the invention contemplates each and every possible

variation of polynucleotide sequence that could be made by selecting combinations based

on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring SIGP, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode SIGP and its variants are preferably
5   capable of hybridizing to the nucleotide sequence of the naturally occurring SIGP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding SIGP or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the
10 frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding SIGP and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

15 The invention also encompasses production of DNA sequences which encode SIGP and SIGP derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence
20 encoding SIGP or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID
25 NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; and Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.)

Methods for DNA sequencing are well known and generally available in the art and may be used to practice any of the embodiments of the invention. The methods may
30 employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp., Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proofreading

exonucleases such as those found in the ELONGASE Amplification System (GIBCO/BRL, Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA Sequencers

5   (Perkin Elmer).

The nucleic acid sequences encoding SIGP may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal primers to retrieve unknown

10   sequence adjacent to a known locus. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) In particular, genomic DNA is first amplified in the presence of a primer complementary to a linker sequence within the vector and a primer specific to the region predicted to encode the gene. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first

15   one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) The primers may be designed using commercially available software such as

20   OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN) or another appropriate program to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to 72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by

25   intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations

30   may be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991)

Nucleic Acids Res. 19:3055-3060.) Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been

5   size-selected to include larger cDNAs. Also, random-primed libraries are preferable in that they will include more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

10   Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and a charge coupled device camera for detection of the emitted wavelengths.

15   Output/light intensity may be converted to electrical signal using appropriate software (e.g., Genotyper™ and Sequence Navigator™, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

20   In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode SIGP may be used in recombinant DNA molecules to direct expression of SIGP, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be

25   produced, and these sequences may be used to clone and express SIGP.

As will be understood by those of skill in the art, it may be advantageous to produce SIGP-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable

30   properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods

generally known in the art in order to alter SIGP-encoding sequences for a variety of reasons including, but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the

5 nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding SIGP may be ligated to a heterologous sequence to encode a

10 fusion protein. For example, to screen peptide libraries for inhibitors of SIGP activity, it may be useful to encode a chimeric SIGP protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the SIGP encoding sequence and the heterologous protein sequence, so that SIGP may be cleaved and purified away from the heterologous moiety.

15   In another embodiment, sequences encoding SIGP may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232.) Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of SIGP, or a fragment thereof. For

20 example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g, Chiez, R.M. and F.Z. Regnier (1990)

25 Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1983) Proteins, Structures and Molecular Properties, WH Freeman and Co., New York, NY.) Additionally, the amino acid sequence of SIGP, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof,

30 to produce a variant polypeptide.

In order to express a biologically active SIGP, the nucleotide sequences encoding SIGP or derivatives thereof may be inserted into appropriate expression vector, i.e., a

vector which contains the necessary elements for the transcription and translation of the
inserted coding sequence.

Methods which are well known to those skilled in the art may be used to construct
expression vectors containing sequences encoding SIGP and appropriate transcriptional
5   and translational control elements. These methods include in vitro recombinant DNA
techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook,
J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press,
Plainview, NY, ch. 4, 8, and 16-17; and Ausubel, F.M. et al. (1995, and periodic
supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York,
10 NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express
sequences encoding SIGP. These include, but are not limited to, microorganisms such as
bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression
vectors; yeast transformed with yeast expression vectors; insect cell systems infected with
15 virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus
expression vectors (e.g., cauliflower mosaic virus (CaMV) or tobacco mosaic virus
(TMV)) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell
systems.
The invention is not limited by the host cell employed.
20   The "control elements" or "regulatory sequences" are those non-translated regions,
e.g., enhancers, promoters, and 5' and 3' untranslated regions, of the vector and
polynucleotide sequences encoding SIGP which interact with host cellular proteins to
carry out transcription and translation. Such elements may vary in their strength and
specificity. Depending on the vector system and host utilized, any number of suitable
25 transcription and translation elements, including constitutive and inducible promoters, may
be used. For example, when cloning in bacterial systems, inducible promoters, e.g., hybrid
lacZ promoter of the Bluescript® phagemid (Stratagene, La Jolla, CA) or pSport1™
plasmid (GIBCO/BRL), may be used. The baculovirus polyhedrin promoter may be used
in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat
30 shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters
or leader sequences) may be cloned into the vector. In mammalian cell systems,
promoters from mammalian genes or from mammalian viruses are preferable. If it is

necessary to generate a cell line that contains multiple copies of the sequence encoding

SIGP, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon

the use intended for SIGP. For example, when large quantities of SIGP are needed for the

5 induction of antibodies, vectors which direct high level expression of fusion proteins that

are readily purified may be used. Such vectors include, but are not limited to,

multifunctional _E. coli_ cloning and expression vectors such as Bluescript® (Stratagene),

in which the sequence encoding SIGP may be ligated into the vector in frame with

sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase so

10 that a hybrid protein is produced, and pIN vectors. (See, e.g., Van Heeke, G. and S.M.

Schuster (1989) J. Biol. Chem. 264:5503-5509.) pGEX vectors (Pharmacia Biotech,

Uppsala, Sweden) may also be used to express foreign polypeptides as fusion proteins

with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can

easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by

15 elution in the presence of free glutathione. Proteins made in such systems may be

designed to include heparin, thrombin, or factor XA protease cleavage sites so that the

cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast _Saccharomyces cerevisiae_, a number of vectors containing constitutive or

inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used. (See,

20 e.g., Ausubel, _supra_; and Grant et al. (1987) Methods Enzymol. 153:516-544.)

In cases where plant expression vectors are used, the expression of sequences

encoding SIGP may be driven by any of a number of promoters. For example, viral

promoters such as the 35S and 19S promoters of CaMV may be used alone or in

combination with the omega leader sequence from TMV. (Takamatsu, N. (1987) EMBO

25 J. 6:307-311.) Alternatively, plant promoters such as the small subunit of RUBISCO or

heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J.

3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991)

Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells

by direct DNA transformation or pathogen-mediated transfection. Such techniques are

30 described in a number of generally available reviews. (See, e.g., Hobbs, S. or Murry, L.E.

in _McGraw Hill Yearbook of Science and Technology_ (1992) McGraw Hill, New York,

NY: pp. 191-196.)

An insect system may also be used to express SIGP. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding SIGP may be cloned into a non-essential region of the virus, such as

5   the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of sequences encoding SIGP will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which SIGP may be expressed. (See, e.g., Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci.

10   91:3224-3227.)

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding SIGP may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the

15   viral genome may be used to obtain a viable virus which is capable of expressing SIGP in infected host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) may also be employed to deliver larger

20   fragments of DNA than can be contained and expressed in a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding SIGP. Such signals include the ATG initiation codon and adjacent

25   sequences. In cases where sequences encoding SIGP and its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon

30   should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers

appropriate for the particular cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such

5   modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding, and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO,

10   HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Bethesda, MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

For long term, high yield production of recombinant proteins, stable expression is preferred. For example, cell lines capable of stably expressing SIGP can be transformed

15   using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and

20   recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase genes and

25   adenine phosphoribosyltransferase genes, which can be employed in tk^- or apr^- cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; and Lowy, I. et al. (1980) Cell 22:817-823) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, dhfr confers resistance to methotrexate; npt confers resistance to the aminoglycosides neomycin and G-418; and als or pat confer

30   resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14; and Murry, supra.) Additional selectable genes have been

described, e.g., *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Recently, the use of visible markers has gained popularity with such markers as anthocyanins, ß glucuronidase and its

5   substrate GUS, luciferase and its substrate luciferin. Green fluorescent proteins (GFP) (Clontech, Palo Alto, CA) are also used (See, e.g., Chalfie, M. et al. (1994) Science 263:802-805.) These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131.)

10   Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding SIGP is inserted within a marker gene sequence, transformed cells containing sequences encoding SIGP can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a

15   sequence encoding SIGP under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain the nucleic acid sequence encoding SIGP and express SIGP may be identified by a variety of procedures known to those of skill in the

20   art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

The presence of polynucleotide sequences encoding SIGP can be detected by

25   DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding SIGP. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding SIGP to detect transformants containing DNA or RNA encoding SIGP.

A variety of protocols for detecting and measuring the expression of SIGP, using

30   either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site,

monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two
non-interfering epitopes on SIGP is preferred, but a competitive binding assay may be
employed. These and other assays are well described in the art. (See, e.g., Hampton, R. et
al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN, Section
5   IV; and Maddox, D.E. et al. (1983) J. Exp. Med. 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the
art and may be used in various nucleic acid and amino acid assays. Means for producing
labeled hybridization or PCR probes for detecting sequences related to polynucleotides
encoding SIGP include oligolabeling, nick translation, end-labeling, or PCR amplification
10   using a labeled nucleotide. Alternatively, the sequences encoding SIGP, or any fragments
thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors
are known in the art, are commercially available, and may be used to synthesize RNA
probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and
labeled nucleotides. These procedures may be conducted using a variety of commercially
15   available kits, such as those provided by Pharmacia & Upjohn (Kalamazoo, Ml), Promega
(Madison, WI), and U.S. Biochemical Corp. (Cleveland, OH). Suitable reporter molecules
or labels which may be used for ease of detection include radionuclides, enzymes,
fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors,
inhibitors, magnetic particles, and the like.

20   Host cells transformed with nucleotide sequences encoding SIGP may be cultured
under conditions suitable for the expression and recovery of the protein from cell culture.
The protein produced by a transformed cell may be secreted or contained intracellularly
depending on the sequence and/or the vector used. As will be understood by those of skill
in the art, expression vectors containing polynucleotides which encode SIGP may be
25   designed to contain signal sequences which direct secretion of SIGP through a prokaryotic
or eukaryotic cell membrane. Other constructions may be used to join sequences encoding
SIGP to nucleotide sequences encoding a polypeptide domain which will facilitate
purification of soluble proteins. Such purification facilitating domains include, but are not
limited to, metal chelating peptides such as histidine-tryptophan modules that allow
30   purification on immobilized metals, protein A domains that allow purification on
immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity
purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker

-45-

sequences, such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA), between the purification domain and the SIGP encoding sequence may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing SIGP and a nucleic acid encoding 6 histidine residues preceding a

5   thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on immobilized metal ion affinity chromatography. (IMAC) (See, e.g., Porath, J. et al. (1992) Prot. Exp. Purif. 3: 263-281.) The enterokinase cleavage site provides a means for purifying SIGP from the fusion protein. (See, e.g., Kroll, D.J. et al. (1993) DNA Cell Biol. 12:441-453.)

10   Fragments of SIGP may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, T.E. (1984) Protein: Structures and Molecular Properties, pp. 55-60, W.H. Freeman and Co., New York, NY.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the Applied Biosystems 431A

15   Peptide Synthesizer (Perkin Elmer). Various fragments of SIGP may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

The expression of the human signal peptide-containing proteins of the invention

20   (SIGP) is closely associated with cell proliferation. Therefore, in cancers or immune response where SIGP is an activator, transcription factor, or enhancer, and is promoting cell proliferation, it is desirable to decrease the expression of SIGP. In conditions where SIGP is an inhibitor or suppressor and is controlling or decreasing cell proliferation, it is desirable to provide the protein or to increase the expression of SIGP.

25   In one embodiment, where SIGP is an inhibitor, SIGP or a fragment or derivative thereof may be administered to a subject to treat or prevent a cancer such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma. Such cancers include, but are not limited to, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal

30   tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

In another embodiment, a pharmaceutical composition comprising purified SIGP may

be used to treat or prevent a cancer including, but not limited to, those listed above.

In another embodiment, an agonist which is specific for SIGP may be administered to a subject to treat or prevent a cancer including, but not limited to, those cancers listed above.

5   In another further embodiment, a vector capable of expressing SIGP, or a fragment or a derivative thereof, may be administered to a subject to treat or prevent a cancer including, but not limited to, those cancers listed above.

In a further embodiment where SIGP is promoting cell proliferation, antagonists which decrease the expression or activity of SIGP may be administered to a subject to treat

10   or prevent a cancer such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma. Such cancers include, but are not limited to, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. In one

15   aspect, antibodies which specifically bind SIGP may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express SIGP.

In another embodiment, a vector expressing the complement of the polynucleotide encoding SIGP may be administered to a subject to treat or prevent a cancer including, but

20   not limited to, those cancers listed above.

In yet another embodiment where SIGP is promoting leukocyte activity or proliferation, antagonists which decrease the activity of SIGP may be administered to a subject to treat or prevent an immune response. Such responses include, but are not limited to, disorders such as AIDS, Addison's disease, adult respiratory distress syndrome,

25   allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitus, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis,

30   rheumatoid arthritis, scleroderma, Sjögren's syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, extracorporeal circulation: viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; and trauma. In one aspect, antibodies

which specifically bind SIGP may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express SIGP.

In another embodiment, a vector expressing the complement of the polynucleotide

5   encoding SIGP may be administered to a subject to treat or prevent an immune response including, but not limited to, those listed above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in

10   combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

15   An antagonist of SIGP may be produced using methods which are generally known in the art. In particular, purified SIGP may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind SIGP. Antibodies to SIGP may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and

20   single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with SIGP or with any fragment or

25   oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli

30   Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to SIGP have an amino acid sequence consisting of at least about 5 amino acids, and, more

preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of SIGP amino acids may be fused with those of another

5   protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to SIGP may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature

10   256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with

15   appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce SIGP-specific single chain antibodies. Antibodies

20   with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding

25   reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; and Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for SIGP may also be generated. For example, such fragments include, but are not limited to, F(ab')2 fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by

30   reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science

246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are

5   well known in the art. Such immunoassays typically involve the measurement of complex formation between SIGP and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering SIGP epitopes is preferred, but a competitive binding assay may also be employed. (Maddox, supra.)

10   In another embodiment of the invention, the polynucleotides encoding SIGP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding SIGP may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding SIGP. Thus,

15   complementary molecules or fragments may be used to modulate SIGP activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding SIGP.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia

20   viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors which will express nucleic acid sequences complementary to the polynucleotides of the gene encoding SIGP. (See, e.g., Sambrook, supra; and Ausubel, supra.)

25   Genes encoding SIGP can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding SIGP. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous

30   nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding SIGP. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site,

5   are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr,

10 Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific

15 hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding SIGP.

Specific ribozyme cleavage sites within any potential RNA target are initially

20 identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be

25 evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase

30 phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding SIGP. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA

polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life.
5   Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as
10   acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous
15   transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nature Biotechnology 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses,
20   rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of SIGP, antibodies to SIGP, and mimetics, agonists,
25   antagonists, or inhibitors of SIGP. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.
30   The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous,

intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used

5   pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of <u>Remington's Pharmaceutical Sciences</u> (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral

10   administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules

15   (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including

20   arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol

25   gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made

30   of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally,

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stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of SIGP, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions

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wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example SIGP or fragments thereof, antibodies of SIGP, and agonists, antagonists or inhibitors of SIGP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED50 (the dose therapeutically effective in 50% of the population) or LD50 (the dose lethal to 50% of the population) statistics. The dose ratio of therapeutic to toxic effects is the therapeutic index, and it can be expressed as the ED50/LD50 ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to

practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

5 **DIAGNOSTICS**

In another embodiment, antibodies which specifically bind SIGP may be used for the diagnosis of disorders characterized by expression of SIGP, or in assays to monitor patients being treated with SIGP or agonists, antagonists, or inhibitors of SIGP. Antibodies useful for diagnostic purposes may be prepared in the same manner as

10 described above for therapeutics. Diagnostic assays for SIGP include methods which utilize the antibody and a label to detect SIGP in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

15 A variety of protocols for measuring SIGP, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of SIGP expression. Normal or standard values for SIGP expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to SIGP under conditions suitable for complex formation The amount of

20 standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of SIGP expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding SIGP may be

25 used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of SIGP may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of SIGP, and to monitor

30 regulation of SIGP levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding SIGP or closely related

molecules may be used to identify nucleic acid sequences which encode SIGP. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding SIGP, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the SIGP encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, or from genomic sequences including promoters, enhancers, and introns of the SIGP gene.

Means for producing specific hybridization probes for DNAs encoding SIGP include the cloning of polynucleotide sequences encoding SIGP or SIGP derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding SIGP may be used for the diagnosis of a disorder associated with either increased or decreased expression of SIGP. Examples of such a disorder include, but are not limited to, cancers such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and cancers of the adrenal gland, bladder, bone, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, bone marrow, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; neuronal disorders such as akathesia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, multiple sclerosis,

neurofibromatosis, Parkinson's disease, paranoid psychoses, schizophrenia, and Tourette's

disorder; and immunological disorders such as AIDS, Addison's disease, adult respiratory

distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitus,

Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus,

5   emphysema, atrophic gastritis, glomerulonephritis, gout, Graves' disease,

hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis,

myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis,

pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, and

thyroiditis. The polynucleotide sequences encoding SIGP may be used in Southern or

10   northern analysis, dot blot, or other membrane-based technologies; in PCR technologies;

in dipstick, pin, and ELISA assays; and in microarrays utilizing fluids or tissues from

patients to detect altered SIGP expression. Such qualitative or quantitative methods are

well known in the art.

In a particular aspect, the nucleotide sequences encoding SIGP may be useful in

15   assays that detect the presence of associated disorders, particularly those mentioned above.

The nucleotide sequences encoding SIGP may be labeled by standard methods and added

to a fluid or tissue sample from a patient under conditions suitable for the formation of

hybridization complexes. After a suitable incubation period, the sample is washed and the

signal is quantitated and compared with a standard value. If the amount of signal in the

20   patient sample is significantly altered in comparison to a control sample then the presence

of altered levels of nucleotide sequences encoding SIGP in the sample indicates the

presence of the associated disorder. Such assays may also be used to evaluate the efficacy

of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to

monitor the treatment of an individual patient.

25   In order to provide a basis for the diagnosis of a disorder associated with expression

of SIGP, a normal or standard profile for expression is established. This may be

accomplished by combining body fluids or cell extracts taken from normal subjects, either

animal or human, with a sequence, or a fragment thereof, encoding SIGP, under conditions

suitable for hybridization or amplification. Standard hybridization may be quantified by

30   comparing the values obtained from normal subjects with values from an experiment in

which a known amount of a substantially purified polynucleotide is used. Standard values

obtained in this manner may be compared with values obtained from samples from

patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of
5 expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of
10 the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding
15 SIGP may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding SIGP, or a fragment of a polynucleotide complementary to the polynucleotide encoding SIGP, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may
20 also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of SIGP include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J.
25 Immunol. Methods 159:235-244; and Duplaa, C. et al. (1993) Anal. Biochem. 229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the
30 polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This

information may be used to determine gene function, to understand the genetic basis of a

disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic

agents.

In one embodiment, the microarray is prepared and used according to methods known

5   in the art. (See, e.g., Chee et al. (1995) PCT application WO95/11995; Lockhart, D. J. et

al. (1996) Nat. Biotech. 14:1675-1680; and Schena, M. et al. (1996) Proc. Natl. Acad. Sci.

93:10614-10619.)

The microarray is preferably composed of a large number of unique single-stranded

nucleic acid sequences, usually either synthetic antisense oligonucleotides or fragments of

10 cDNAs. The oligonucleotides are preferably about 6 to 60 nucleotides in length, more

preferably about 15 to 30 nucleotides in length, and most preferably about 20 to 25

nucleotides in length. It may be preferable to use oligonucleotides which are about 7 to 10

nucleotides in length. The microarray may contain oligonucleotides which cover the

known 5' or 3' sequence, sequential oligonucleotides which cover the full length sequence,

15 or unique oligonucleotides selected from particular areas along the length of the sequence.

Polynucleotides used in the microarray may be oligonucleotides specific to a gene or genes

of interest. Oligonucleotides can also be specific to one or more unidentified cDNAs

associated with a particular cell type or tissue type. It may be appropriate to use pairs of

oligonucleotides on a microarray. The first oligonucleotide in each pair differs from the

20 second oligonucleotide by one nucleotide. This nucleotide is preferably located in the

center of the sequence. The second oligonucleotide serves as a control. The number of

oligonucleotide pairs may range from about 2 to 1,000,000.

In order to produce oligonucleotides for use on a microarray, the gene of interest is

examined using a computer algorithm which starts at the 5' end, or, more preferably, at the

25 3' end of the nucleotide sequence. The algorithm identifies oligomers of defined length

that are unique to the gene, have a GC content within a range suitable for hybridization,

and lack secondary structure that may interfere with hybridization. In one aspect, the

oligomers may be synthesized on a substrate using a light-directed chemical process. (See.

e.g., Chee et al., supra.) The substrate may be any suitable solid support. e.g., paper.

30 nylon, any other type of membrane, or a filter, chip, or glass slide.

In another aspect, the oligonucleotides may be synthesized on the surface of the

substrate using a chemical coupling procedure and an ink jet application apparatus. (See,

e.g., Baldeschweiler et al. (1995) PCT application WO95/251116.) An array analogous to a dot or slot blot (HYBRIDOT® apparatus, GIBCO/BRL) may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system or thermal, UV, mechanical, or chemical bonding procedures. An array may also be

5   produced by hand or by using available devices, materials, and machines, e.g. Brinkmann® multichannel pipettors or robotic instruments. The array may contain from 2 to 1,000,000 or any other feasible number of oligonucleotides.

    In order to conduct sample analysis using the microarrays, polynucleotides are extracted from a sample. The sample may be obtained from any bodily fluid, e.g., blood,

10   urine, saliva, phlegm, gastric juices, cultured cells, biopsies, or other tissue preparations. To produce probes, the polynucleotides extracted from the sample are used to produce nucleic acid sequences complementary to the nucleic acids on the microarray. If the microarray contains cDNAs, antisense RNAs (aRNAs) are appropriate probes. Therefore, in one aspect, mRNA is reverse-transcribed to cDNA. The cDNA, in the presence of

15   fluorescent label, is used to produce fragment or oligonucleotide aRNA probes. The fluorescently labeled probes are incubated with the microarray so that the probes hybridize to the microarray oligonucleotides. Nucleic acid sequences used as probes can include polynucleotides, fragments, and complementary or antisense sequences produced using restriction enzymes, PCR, or other methods known in the art.

20   Hybridization conditions can be adjusted so that hybridization occurs with varying degrees of complementarity. A scanner can be used to determine the levels and patterns of fluorescence after removal of any nonhybridized probes. The degree of complementarity and the relative abundance of each oligonucleotide sequence on the microarray can be assessed through analysis of the scanned images. A detection system may be used to

25   measure the absence, presence, or level of hybridization for any of the sequences. (See, e.g., Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155.)

    In another embodiment of the invention, nucleic acid sequences encoding SIGP may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a

30   specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries.

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(See, e.g., Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al.

5   (1995) in Meyers, R.A. (ed.) Molecular Biology and Biotechnology, VCH Publishers New York, NY, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding SIGP on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA

10 associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another

15 mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to

20 a particular genomic region, e.g., AT to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

25 In another embodiment of the invention, SIGP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between SIGP and the agent being

30 tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen,

et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with SIGP, or fragments thereof, and washed. Bound SIGP is then detected by methods well known in the art. Purified SIGP

5   can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding SIGP specifically compete with a test

10 compound for binding SIGP. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with SIGP.

In additional embodiments, the nucleotide sequences which encode SIGP may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including,

15 but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

20   **EXAMPLES**

For purposes of example, the preparation and sequencing of the SPLNNOT04 cDNA library, from which Incyte Clones 1534876 and 1559131 were isolated, is described. Preparation and sequencing of cDNAs in libraries in the LIFESEQ™ database have varied over time, and the gradual changes involved use of kits, plasmids, and machinery available

25 at the particular time the library was made and analyzed.

I.   **SPLNNOT04 cDNA Library Construction**

The SPLNNOT04 cDNA library was constructed from microscopically normal spleen tissue obtained from a 2-year-old Hispanic male who died of cerebral anoxia. The

30 patient's serologies and past medical history were negative.

The frozen tissue was homogenized and lysed using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury, NJ) in guanidinium isothiocyanate

solution. The lysate was centrifuged over a 5.7 M CsCl cushion using an Beckman SW28 rotor in a Beckman L8-70M Ultracentrifuge (Beckman Instruments) for 18 hours at 25,000 rpm at ambient temperature. The RNA was extracted with acid phenol pH 4.0, precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in

5   RNAse-free water and DNase treated at 37°C. The RNA extraction and precipitation were repeated as before. The mRNA was then isolated using the Qiagen Oligotex kit (QIAGEN Inc., Chatsworth, CA) and used to construct the cDNA library.

The mRNA was handled according to the recommended protocols in the SuperScript plasmid system (Cat. #18248-013, GIBCO-BRL, Gaithersburg, MD). cDNA synthesis was

10   initiated with a NotI-oligo d(T) primer. Double-stranded cDNA was blunted, ligated to EcoRI adaptors, digested with NotI, fractionated on a Sepharose CL4B column (Cat. #275105-01, Pharmacia), and those cDNAs exceeding 400 bp were ligated into the NotI and EcoRI sites of the pINCY 1 vector (Incyte). The plasmid pINCY 1 was subsequently transformed into DH5α™ competent cells (Cat. #18258-012, GIBCO-BRL).

15

II   Isolation and Sequencing of cDNA Clones

Plasmid cDNA was released from the cells and purified using the REAL Prep 96 plasmid kit (Catalog #26173, QIAGEN). The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile Terrific

20   Broth (Catalog #22711, GIBCO-BRL) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) after inoculation, the cultures were incubated for 19 hours and at the end of incubation, the cells were lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellet was resuspended in 0.1 ml of distilled water. After the last step in the protocol, samples were transferred to a 96-well block for storage at 4° C.

25   cDNAs were sequenced according to the method of Sanger et al. (1975, J. Mol. Biol. 94:441f), using the Perkin Elmer Catalyst 800 or a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown, MA) and Applied Biosystems 377 DNA Sequencing Systems or the Perkin Elmer 373 DNA Sequencing System and the reading frame was determined.

30

III. Homology Searching of cDNA Clones and Their Deduced Proteins

The nucleotide sequences and/or amino acid sequences of the Sequence Listing were

used to query sequences in the GenBank, SwissProt, BLOCKS, and Pima II databases. These databases, which contain previously identified and annotated sequences, were searched for regions of homology using BLAST (Basic Local Alignment Search Tool). (See, e.g., Altschul, S.F. (1993) J. Mol. Evol 36:290-300; and Altschul et al. (1990) J.

5　Mol. Biol. 215:403-410.)

BLAST produced alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST was especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal, or plant) origin. Other algorithms

10　could have been used when dealing with primary sequence patterns and secondary structure gap penalties. (See, e.g., Smith, T. et al. (1992) Protein Engineering 5:35-51.) The sequences disclosed in this application have lengths of at least 49 nucleotides and have no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach searched for matches between a query sequence and a database

15　sequence. BLAST evaluated the statistical significance of any matches found, and reported only those matches that satisfy the user-selected threshold of significance. In this application, threshold was set at 10^{-25} for nucleotides and 10^{-8} for peptides.

Incyte nucleotide sequences were searched against the GenBank databases for primate (pri), rodent (rod), and other mammalian sequences (mam), and deduced amino acid

20　sequences from the same clones were then searched against GenBank functional protein databases, mammalian (mamp), vertebrate (vrtp), and eukaryote (eukp), for homology.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript

25　of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; and Ausubel, F.M. et al. supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST are used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ™ database

30　(Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score, which is defined as:

% sequence identity x % maximum BLAST score

100

The product score takes into account both the degree of similarity between two sequences
5   and the length of the sequence match. For example, with a product score of 40, the match
will be exact within a 1% to 2% error, and, with a product score of 70, the match will be
exact. Homologous molecules are usually identified by selecting those which show
product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the
10   transcript encoding SIGP occurs. Abundance and percent abundance are also reported.
Abundance directly reflects the number of times a particular transcript is represented in a
cDNA library, and percent abundance is abundance divided by the total number of
sequences examined in the cDNA library.

15   **V.   Extension of SIGP Encoding Polynucleotides**

The nucleic acid sequence of one of the polynucleotides of the present invention was
used to design oligonucleotide primers for extending a partial nucleotide sequence to full
length. One primer was synthesized to initiate extension of an antisense polynucleotide,
and the other was synthesized to initiate extension of a sense polynucleotide. Primers
20   were used to facilitate the extension of the known sequence "outward" generating
amplicons containing new unknown nucleotide sequence for the region of interest. The
initial primers were designed from the cDNA using OLIGO 4.06 (National Biosciences,
Plymouth, MN), or another appropriate program, to be about 22 to 30 nucleotides in
length, to have a GC content of about 50% or more, and to anneal to the target sequence at
25   temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result
in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (GIBCO/BRL) were used to extend the sequence. If
more than one extension is necessary or desired, additional sets of primers are designed to
further extend the known region.

30   High fidelity amplification was obtained by following the instructions for the XL-
PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix. PCR was
performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA),

beginning with 40 pmol of each primer and the recommended concentrations of all other

components of the kit, with the following parameters:

	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
5	Step 3	68° C for 6 min
	Step 4	94° C for 15 sec
	Step 5	65° C for 1 min
	Step 6	68° C for 7 min
	Step 7	Repeat steps 4 through 6 for an additional 15 cycles
10	Step 8	94° C for 15 sec
	Step 9	65° C for 1 min
	Step 10	68° C for 7:15 min
	Step 11	Repeat steps 8 through 10 for an additional 12 cycles
	Step 12	72° C for 8 min
15	Step 13	4° C (and holding)

A 5 μl to 10 μl aliquot of the reaction mixture was analyzed by electrophoresis on

a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions

were successful in extending the sequence. Bands thought to contain the largest products

20 were excised from the gel, purified using QIAQuick™ (QIAGEN Inc., Chatsworth, CA),

and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13 μl of ligation

buffer, 1μl T4-DNA ligase (15 units) and 1μl T4 polynucleotide kinase were added, and

the mixture was incubated at room temperature for 2 to 3 hours, or overnight at 16° C.

25 Competent E. coli cells (in 40 μl of appropriate media) were transformed with 3 μl of

ligation mixture and cultured in 80 μl of SOC medium. (See, e.g., Sambrook, supra,

Appendix A, p. 2.) After incubation for one hour at 37° C, the E. coli mixture was plated

on Luria Bertani (LB) agar (See, e.g., Sambrook, supra, Appendix A, p. 1) containing 2x

Carb. The following day, several colonies were randomly picked from each plate and

30 cultured in 150 μl of liquid LB/2x Carb medium placed in an individual well of an

appropriate commercially-available sterile 96-well microtiter plate. The following day, 5

μl of each overnight culture was transferred into a non-sterile 96-well plate and, after

dilution 1:10 with water, 5 μl from each sample was transferred into a PCR array.

For PCR amplification, 18 μl of concentrated PCR reaction mix (3.3x) containing

35 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific

primers used for the extension reaction were added to each well. Amplification was

performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec
Step 3	55° C for 30 sec
Step 4	72° C for 90 sec
Step 5	Repeat steps 2 through 4 for an additional 29 cycles
Step 6	72° C for 180 sec
Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the nucleotide sequence of one of the nucleotide sequences of the present invention were used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

VI.   Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from one of the nucleotide sequences of the present invention are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham, Chicago, IL), and T4 polynucleotide kinase (DuPont NEN$^{®}$, Boston, MA). The labeled oligonucleotides are substantially purified using a Sephadex G-25 superfine resin column (Pharmacia & Upjohn, Kalamazoo, MI). An aliquot containing 10^{7} counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba 1, or Pvu II (DuPont NEN, Boston, MA).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film

(Kodak, Rochester, NY) is exposed to the blots to film for several hours, hybridization patterns are compared visually.

VII.   Microarrays

5   To produce oligonucleotides for a microarray, one of the nucleotide sequences of the present invention is examined using a computer algorithm which starts at the 3' end of the nucleotide sequence. For each, the algorithm identifies oligomers of defined length that are unique to the nucleic acid sequence, have a GC content within a range suitable for hybridization, and lack secondary structure that would interfere with hybridization. The

10   algorithm identifies approximately 20 oligonucleotides corresponding to each nucleic acid sequence. For each sequence-specific oligonucleotide, a pair of oligonucleotides is synthesized in which the first oligonucleotides differs from the second oligonucleotide by one nucleotide in the center of the sequence. The oligonucleotide pairs can be arranged on a substrate, e.g. a silicon chip, using a light-directed chemical process. (See, e.g., Chee,

15   supra.)

        In the alternative, a chemical coupling procedure and an ink jet device can be used to synthesize oligomers on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link fragments or oligonucleotides to the surface of a substrate using or thermal, UV, mechanical, or

20   chemical bonding procedures, or a vacuum system. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each oligonucleotide sequence on the microarray may be assessed

25   through analysis of the scanned images.

VIII.   Complementary Polynucleotides

        Sequences complementary to the SIGP-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring SIGP. Although

30   use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using Oligo 4.06 software and the coding sequence of SIGP.

To inhibit transcription, a complementary oligonucleotide is designed from the most
unique 5' sequence and used to prevent promoter binding to the coding sequence. To
inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal
binding to the SIGP-encoding transcript.

5

IX.   Expression of SIGP

Expression of SIGP is accomplished by subcloning the cDNA into an appropriate
vector and transforming the vector into host cells. This vector contains an appropriate
promoter, e.g., ß-galactosidase upstream of the cloning site, operably associated with the

10   cDNA of interest. (See, e.g.,Sambrook, supra, pp. 404-433; and Rosenberg, M. et al.
(1983) Methods Enzymol. 101:123-138.)

Induction of an isolated, transformed bacterial strain with isopropyl beta-D-
thiogalactopyranoside (IPTG) using standard methods produces a fusion protein which
consists of the first 8 residues of ß-galactosidase, about 5 to 15 residues of linker, and the

15   full length protein. The signal residues direct the secretion of SIGP into bacterial growth
media which can be used directly in the following assay for activity.

X.   Production of SIGP Specific Antibodies

SIGP substantially purified using PAGE electrophoresis (see, e.g., Harrington,

20   M.G. (1990) Methods Enzymol. 182:488-495), or other purification techniques, is used to
immunize rabbits and to produce antibodies using standard protocols. The SIGP amino
acid sequence is analyzed using DNASTAR software (DNASTAR Inc) to determine
regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used
to raise antibodies by means known to those of skill in the art. Methods for selection of

25   appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well
described in the art. (See, e.g., Ausubel et al. supra, ch. 11.)

Typically, the oligopeptides are 15 residues in length, and are synthesized using
an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and
coupled to KLH (Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-

30   hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel et al.
supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's
adjuvant. Resulting antisera are tested for antipeptide activity, for example, by binding the

peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XI.   Purification of Naturally Occurring SIGP Using Specific Antibodies

5   Naturally occurring or recombinant SIGP is substantially purified by immunoaffinity chromatography using antibodies specific for SIGP. An immunoaffinity column is constructed by covalently coupling anti-SIGP antibody to an activated chromatographic resin, such as CNBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

10   Media containing SIGP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of SIGP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/SIGP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and SIGP is collected.

15

XII.   Identification of Molecules Which Interact with SIGP

SIGP, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled SIGP,

20   washed, and any wells with labeled SIGP complex are assayed. Data obtained using different concentrations of SIGP are used to calculate values for the number, affinity, and association of SIGP with the candidate molecules.

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and

25   spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following

30   claims.

What is claimed is:

1.   A substantially purified human signal peptide-containing protein (SIGP) comprising a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15.

2.   An isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding an SIGP of claim 1.

3.   An isolated and purified polynucleotide encoding the SIGP of claim 1.

4.   A microarray containing at least a fragment of at least one of the polynucleotides encoding an SIGP of claim 1.

5.   An isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide of claim 3.

6.   A composition comprising the polynucleotide of claim 3.

7.   An isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide of claim 3.

8.   An isolated and purified polynucleotide which is complementary to the polynucleotide of claim 3.

9.   An isolated and purified polynucleotide having a nucleic acid sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30.

10.   An isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide of claim 9.

11.   An isolated and purified polynucleotide which is complementary to the polynucleotide sequence of claim 9.

12.   An expression vector containing at least a fragment of the polynucleotide of claim 3.

13.   A host cell containing the expression vector of claim 12.

14.   A method for producing a polypeptide encoding a human signal peptide-containing protein, the method comprising the steps of:

(a)   culturing the host cell of claim 13 under conditions suitable for the
expression of the polypeptide; and

(b)   recovering the polypeptide from the host cell culture.

15.   A pharmaceutical composition comprising the SIGP of claim 1 in
conjunction with a suitable pharmaceutical carrier.

16.   A purified antibody which specifically binds to the SIGP of claim 1.

17.   A purified agonist of the SIGP of claim 1.

18.   A purified antagonist of the SIGP of claim 1.

19.   A method for treating or preventing a cancer, the method comprising
administering to a subject in need of such treatment an effective amount of the
pharmaceutical composition of claim 15.

20.   A method for treating or preventing a cancer, the method comprising
administering to a subject in need of such treatment an effective amount of the antagonist
of claim 18.

21.   A method for treating or preventing an immune response, the method
comprising administering to a subject in need of such treatment an effective amount of the
antagonist of claim 18.

22.   A method for detecting a polynucleotide encoding a human signal peptide-
containing protein in a biological sample containing nucleic acids, the method comprising
the steps of:

(a)   hybridizing the polynucleotide of claim 8 to at least one of the
nucleic acids of the biological sample, thereby forming a hybridization complex;
and

(b)   detecting the hybridization complex, wherein the presence of the
hybridization complex correlates with the presence of a polynucleotide encoding
SIGP in the biological sample.

23.   The method of claim 22 wherein the nucleic acids of the biological sample
are amplified by the polymerase chain reaction prior to the hybridizing step.

SEQUENCE LISTING

<110> INCYTE PHARMACEUTICALS, INC.
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       BAUGHN, Mariah R.
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Lys Val Cys Gly Leu Arg Ala Pro His Gln Pro Ala Pro Cys Ser
                95                  100                 105
Thr Gly Pro Pro Leu Gly Arg Val Pro Ser Leu Arg Pro Pro Pro
                110                 115                 120
Arg Pro Pro Arg Arg Leu Pro His Pro Ser Ser Ile Ser Cys Leu
                125                 130                 135
Glu Arg Leu Trp Thr Leu Gly Pro Pro Ser Pro Ala Thr Arg Arg
                140                 145                 150
Leu Glu Ser Arg Cys Pro Ala Pro Ala Ala Thr Pro Pro Ser Thr
                155                 160                 165
Pro Pro Pro Arg Xaa Xaa Phe Lys Gly Cys Lys Asn
                170                 175


<210> 10
<211> 197
<212> PRT
<213> Homo sapiens

<220> -
<223> 1880830

<400> 10
Met Ile Thr Cys Arg Val Cys Gln Ser Leu Ile Asn Val Glu Gly
 1               5                  10                  15
Lys Met His Gln His Val Val Lys Cys Gly Val Cys Asn Glu Ala
                20                  25                  30
Thr Pro Ile Lys Asn Ala Pro Pro Gly Lys Lys Tyr Val Arg Cys
                35                  40                  45
Pro Cys Asn Cys Leu Leu Ile Cys Lys Val Thr Ser Gln Arg Ile
                50                  55                  60
Ala Cys Pro Arg Pro Tyr Cys Lys Arg Ile Ile Asn Leu Gly Pro
```

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                    65                    70                    75
Val His Pro Gly Pro Leu Ser Pro Glu Pro Gln Pro Met Gly Val
                    80                    85                    90
Arg Val Ile Cys Gly His Cys Lys Asn Thr Phe Leu Trp Thr Glu
                    95                   100                   105
Phe Thr Asp Arg Thr Leu Ala Arg Cys Pro His Cys Arg Lys Val
                   110                   115                   120
Ser Ser Ile Gly Arg Arg Tyr Pro Arg Lys Arg Cys Ile Cys Cys
                   125                   130                   135
Phe Leu Leu Gly Leu Leu Leu Ala Val Thr Ala Thr Gly Leu Ala
                   140                   145                   150
Phe Gly Thr Trp Lys His Ala Arg Arg Tyr Gly Gly Ile Tyr Ala
                   155                   160                   165
Ala Trp Ala Phe Val Ile Leu Leu Ala Val Leu Cys Leu Gly Arg
                   170                   175                   180
Ala Leu Tyr Trp Ala Cys Met Lys Val Ser His Pro Val Gln Asn
                   185                   190                   195
Phe Ser


<210> 11
<211> 346
<212> PRT
<213> Homo sapiens

<220> -
<223> 2328134

<400> 11
Met Thr Pro Arg Thr Trp Trp Pro Arg Pro Ala Gly Trp Gly Thr
 1               5                    10                    15
Cys Arg Ala Ala Gly Trp Pro Arg Ser Val Pro Trp Ala Arg Thr
                20                    25                    30
Ala Ala Ser Leu Val Phe Val Pro Thr Arg Arg Arg Ser Gly Pro
                35                    40                    45
Ser Gly Thr Ala Ser Val Ala Ala Met Ala Tyr His Ser Gly Tyr
                50                    55                    60
Gly Ala His Gly Ser Lys His Arg Ala Arg Ala Ala Pro Asp Pro
                65                    70                    75
Pro Pro Leu Phe Asp Asp Thr Ser Gly Gly Tyr Ser Ser Gln Pro
                80                    85                    90
Gly Gly Tyr Pro Ala Thr Gly Ala Asp Val Ala Phe Ser Val Asn
                95                   100                   105
His Leu Leu Gly Asp Pro Met Ala Asn Val Ala Met Ala Tyr Gly
               110                   115                   120
Ser Ser Ile Ala Ser His Gly Lys Asp Met Val His Lys Glu Leu
               125                   130                   135
His Arg Phe Val Ser Val Ser Lys Leu Lys Tyr Phe Phe Ala Val
               140                   145                   150
Asp Thr Ala Tyr Val Ala Lys Lys Leu Gly Leu Leu Val Phe Pro
               155                   160                   165
Tyr Thr His Gln Asn Trp Glu Val Gln Tyr Ser Arg Asp Ala Pro
               170                   175                   180
Leu Pro Pro Arg Gln Asp Leu Asn Ala Pro Asp Leu Tyr Ile Pro
               185                   190                   195
Thr Met Ala Phe Ile Thr Tyr Val Leu Leu Ala Gly Met Ala Leu
```

```
                     200                    205                    210
Gly Ile Gln Lys Arg Phe Ser Pro Glu Val Leu Gly Leu Cys Ala
                     215                    220                    225
Ser Thr Ala Leu Val Trp Val Val Met Glu Val Leu Ala Leu Leu
                     230                    235                    240
Leu Gly Leu Tyr Leu Ala Thr Val Arg Ser Asp Leu Ser Thr Phe
                     245                    250                    255
His Leu Leu Ala Tyr Ser Gly Tyr Lys Tyr Val Gly Met Ile Leu
                     260                    265                    270
Ser Val Leu Thr Gly Leu Leu Phe Gly Ser Asp Gly Tyr Tyr Val
                     275                    280                    285
Ala Leu Ala Trp Thr Ser Ser Ala Leu Met Tyr Phe Ile Val Arg
                     290                    295                    300
Ser Leu Arg Thr Ala Ala Leu Gly Pro Asp Ser Met Gly Gly Pro
                     305                    310                    315
Val Pro Arg Gln Arg Leu Gln Leu Tyr Leu Thr Leu Gly Ala Ala
                     320                    325                    330
Ala Phe Gln Pro Leu Ile Ile Tyr Trp Leu Thr Phe His Leu Val
                     335                    340                    345
Arg
```

```
<210> 12
<211> 256
<212> PRT
<213> Homo sapiens

<220> -
<223> 2652271

<400> 12
Met Arg Pro Ala Ala Leu Arg Gly Ala Leu Leu Gly Cys Leu Cys
1               5                   10                     15
Leu Ala Leu Leu Cys Leu Gly Gly Ala Asp Lys Arg Leu Arg Asp
                20                  25                     30
Asn His Glu Trp Lys Lys Leu Ile Met Val Gln His Trp Pro Glu
                35                  40                     45
Thr Val Cys Glu Lys Ile Gln Asn Asp Cys Arg Asp Pro Pro Asp
                50                  55                     60
Tyr Trp Thr Ile His Gly Leu Trp Pro Asp Lys Ser Glu Gly Cys
                65                  70                     75
Asn Arg Ser Trp Pro Phe Asn Leu Glu Glu Ile Lys Asp Leu Leu
                80                  85                     90
Pro Glu Met Arg Ala Tyr Trp Pro Asp Val Ile His Ser Phe Pro
                95                  100                    105
Asn Arg Ser Arg Phe Trp Lys His Glu Trp Glu Lys His Gly Thr
                110                 115                    120
Cys Ala Ala Gln Val Asp Ala Leu Asn Ser Gln Lys Lys Tyr Phe
                125                 130                    135
Gly Arg Ser Leu Glu Leu Tyr Arg Glu Leu Asp Leu Asn Ser Val
                140                 145                    150
Leu Leu Lys Leu Gly Ile Lys Pro Ser Ile Asn Tyr Tyr Gln Val
                155                 160                    165
Ala Asp Phe Lys Asp Ala Leu Ala Arg Val Tyr Gly Val Ile Pro
                170                 175                    180
```

```
Lys Ile Gln Cys Leu Pro Pro Ser Gln Asp Glu Glu Val Gln Thr
              185             190             195
Ile Gly Gln Ile Glu Leu Cys Leu Thr Lys Gln Asp Gln Gln Leu
              200             205             210
Gln Asn Cys Thr Glu Pro Gly Glu Gln Pro Ser Pro Lys Gln Glu
              215             220             225
Val Trp Leu Ala Asn Gly Ala Ala Glu Ser Arg Gly Leu Arg Val
              230             235             240
Cys Glu Asp Gly Pro Val Phe Tyr Pro Pro Pro Lys Lys Thr Lys
              245             250             255
His
```

```
<210> 13
<211> 235
<212> PRT
<213> Homo sapiens

<220> -
<223> 2965248

<400> 13
Met Ala Ser Thr Ile Ser Ala Tyr Lys Glu Lys Met Lys Glu Leu
  1             5              10              15
Ser Val Leu Ser Leu Ile Cys Ser Cys Phe Tyr Thr Gln Pro His
              20              25              30
Pro Asn Thr Val Tyr Gln Tyr Gly Asp Met Glu Val Lys Gln Leu
              35              40              45
Asp Lys Arg Ala Ser Gly Gln Ser Phe Glu Val Ile Leu Lys Ser
              50              55              60
Pro Ser Asp Leu Ser Pro Glu Ser Pro Met Leu Ser Ser Pro Pro
              65              70              75
Lys Lys Lys Asp Thr Ser Leu Glu Glu Leu Gln Lys Arg Leu Glu
              80              85              90
Ala Ala Glu Glu Arg Arg Lys Thr Gln Glu Ala Gln Val Leu Lys
              95             100             105
Gln Leu Ala Asp Gly Ala Ser Thr Ser Ala Arg Cys Cys Thr Arg
              110             115             120
Arg Trp Arg Arg Ile Thr Thr Ser Ala Ala Arg Arg Arg Arg Ser
              125             130             135
Ser Thr Thr Arg Trp Ser Ser Ala Arg Arg Ser Ala Arg His Thr
              140             145             150
Trp Pro His Cys Ala Ser Gly Cys Ala Arg Arg Ser Cys Thr Arg
              155             160             165
Pro Arg Cys Ala Gly Thr Arg Ser Ser Glu Lys Arg Cys Arg Ala
              170             175             180
Lys Gly Pro Gly Arg Ala Ala Pro Ile Leu Arg Arg Asn Thr Phe
              185             190             195
Gly Phe Trp Phe Cys Phe Val His Leu Cys Leu Asp Ala Thr Phe
              200             205             210
Val Pro Pro Pro Pro Gln Pro Pro Ala Ser Cys Phe Ser Ser
              215             220             225
Ala Leu Ser Arg Pro Ala Leu Ser Ser Trp
              230             235
```

<210> 14
<211> 371
<212> PRT
<213> Homo sapiens

<220> -
<223> 3057669

<400> 14

```
Met Asp His Glu Asp Ile Ser Glu Ser Val Asp Ala Ala Tyr Asn
1               5                   10                  15
Leu Gln Asp Ser Cys Leu Thr Asp Cys Asp Val Glu Asp Gly Thr
                20                  25                  30
Met Asp Gly Asn Asp Glu Gly His Ser Phe Glu Leu Cys Pro Ser
                35                  40                  45
Glu Ala Ser Pro Tyr Val Arg Ser Arg Glu Arg Thr Ser Ser Ser
                50                  55                  60
Ile Val Phe Glu Asp Ser Gly Cys Asp Asn Ala Ser Ser Lys Glu
                65                  70                  75
Glu Pro Lys Thr Asn Arg Leu His Ile Gly Asn His Cys Ala Asn
                80                  85                  90
Lys Leu Thr Ala Phe Lys Pro Thr Ser Ser Lys Ser Ser Ser Glu
                95                  100                 105
Ala Thr Leu Ser Ile Ser Pro Pro Arg Pro Thr Thr Leu Ser Leu
                110                 115                 120

Asp Leu Thr Lys Asn Thr Thr Glu Lys Leu Gln Pro Ser Ser Pro
                125                 130                 135
Lys Val Tyr Leu Tyr Ile Gln Met Gln Leu Cys Arg Lys Glu Asn
                140                 145                 150
Leu Lys Asp Trp Met Asn Gly Arg Cys Thr Ile Glu Glu Arg Glu
                155                 160                 165
Arg Ser Val Cys Leu His Ile Phe Leu Gln Ile Ala Glu Ala Val
                170                 175                 180
Glu Phe Leu His Ser Lys Gly Leu Met His Arg Asp Leu Lys Pro
                185                 190                 195
Ser Asn Ile Phe Phe Thr Met Asp Asp Val Val Lys Val Gly Asp
                200                 205                 210
Phe Gly Leu Val Thr Ala Met Asp Gln Asp Glu Glu Glu Gln Thr
                215                 220                 225
Val Leu Thr Pro Met Pro Ala Tyr Ala Arg His Thr Gly Gln Val
                230                 235                 240
Gly Thr Lys Leu Tyr Met Ser Pro Glu Gln Ile His Gly Asn Ser
                245                 250                 255
Tyr Ser His Lys Val Asp Ile Phe Ser Leu Gly Leu Ile Leu Phe
                260                 265                 270
Glu Leu Leu Tyr Pro Phe Ser Thr Gln Met Glu Arg Val Arg Thr
                275                 280                 285
Leu Thr Asp Val Arg Asn Leu Lys Phe Pro Pro Leu Phe Thr Gln
                290                 295                 300
Lys Tyr Pro Cys Glu Tyr Val Met Val Gln Asp Met Leu Ser Pro
                305                 310                 315
Ser Pro Met Glu Arg Pro Glu Ala Ile Asn Ile Ile Glu Asn Ala
                320                 325                 330
Val Phe Glu Asp Leu Asp Phe Pro Gly Lys Thr Val Leu Arg Gln
                335                 340                 345
```

```
Arg Ser Arg Ser Leu Ser Ser Ser Gly Thr Lys His Ser Arg Gln
            350             355             360
Ser Asn Asn Ser His Ser Pro Leu Pro Ser Asn
            365             370
```

```
<210> 15
<211> 523
<212> PRT
<213> Homo sapiens

<220> -
<223> 3125156

<400> 15
Met Gly Pro Gln Ala Ala Pro Leu Thr Ile Arg Gly Pro Ser Ser
 1             5              10             15
Ala Gly Gln Ser Thr Pro Ser Pro His Leu Val Pro Ser Pro Ala
            20             25             30
Pro Ser Pro Gly Pro Gly Pro Val Pro Pro Arg Pro Pro Ala Ala
            35             40             45
Glu Pro Pro Pro Cys Leu Arg Arg Gly Ala Ala Ala Ala Asp Leu
            50             55             60
Leu Ser Ser Ser Pro Glu Ser Gln His Gly Gly Thr Gln Ser Pro
            65             70             75
Gly Gly Gly Gln Pro Leu Leu Gln Pro Thr Lys Val Asp Ala Ala
            80             85             90

Glu Gly Arg Arg Pro Gln Ala Leu Arg Leu Ile Glu Arg Asp Pro
            95             100            105
Tyr Glu His Pro Glu Arg Leu Arg Gln Leu Gln Gln Glu Leu Glu
            110            115            120
Ala Phe Arg Gly Gln Leu Gly Asp Val Gly Ala Leu Asp Thr Val
            125            130            135
Trp Arg Glu Leu Gln Asp Ala Gln Glu His Asp Ala Arg Gly Arg
            140            145            150
Ser Ile Ala Ile Ala Arg Cys Tyr Ser Leu Lys Asn Arg His Gln
            155            160            165
Asp Val Met Pro Tyr Asp Ser Asn Arg Val Val Leu Arg Ser Gly
            170            175            180
Lys Asp Asp Tyr Ile Asn Ala Ser Cys Val Glu Gly Leu Ser Pro
            185            190            195
Tyr Cys Pro Pro Leu Val Ala Thr Gln Ala Pro Leu Pro Gly Thr
            200            205            210
Ala Ala Asp Phe Trp Leu Met Val His Glu Gln Lys Val Ser Val
            215            220            225
Ile Val Met Leu Val Ser Glu Ala Glu Met Glu Lys Gln Lys Val
            230            235            240
Ala Arg Tyr Phe Pro Thr Glu Arg Gly Gln Pro Met Val His Gly
            245            250            255
Ala Leu Ser Leu Ala Leu Ser Ser Val Arg Ser Thr Glu Thr His
            260            265            270
Val Glu Arg Val Leu Ser Leu Gln Phe Arg Asp Gln Ser Leu Lys
            275            280            285
Arg Ser Leu Val His Leu His Phe Pro Thr Trp Pro Glu Leu Gly
            290            295            300
```

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```
Leu Pro Asp Ser Pro Ser Asn Leu Leu Arg Phe Ile Gln Glu Val
            305             310                 315
His Ala His Tyr Leu His Gln Arg Pro Leu His Thr Pro Ile Ile
            320             325                 330
Val His Cys Ser Ser Gly Val Gly Arg Thr Gly Ala Phe Ala Leu
            335             340                 345
Leu Tyr Ala Ala Val Gln Glu Val Glu Ala Gly Asn Gly Ile Pro
            350             355                 360
Glu Leu Pro Gln Leu Val Arg Arg Met Arg Gln Gln Arg Lys His
            365             370                 375
Met Leu Gln Glu Lys Leu His Leu Arg Phe Cys Tyr Glu Ala Val
            380             385                 390
Val Arg His Val Glu Gln Val Leu Gln Arg His Gly Val Pro Pro
            395             400                 405
Pro Cys Lys Pro Leu Ala Ser Ala Ser Ile Ser Gln Lys Asn His
            410             415                 420
Leu Pro Gln Asp Ser Gln Asp Leu Val Leu Gly Gly Asp Val Pro
            425             430                 435
Ile Ser Ser Ile Gln Ala Thr Ile Ala Lys Leu Ser Ile Arg Pro
            440             445                 450
Pro Gly Gly Leu Glu Ser Pro Val Ala Ser Leu Pro Gly Pro Ala
            455             460                 465
Glu Pro Pro Gly Leu Pro Pro Ala Ser Leu Pro Glu Ser Thr Pro
            470             475                 480
Ile Pro Ser Ser Ser Gln Thr Pro Phe Pro Pro His Tyr Leu Arg
            485             490                 495
Leu Pro Ser Leu Arg Arg Ser Arg Gln Cys Leu Lys Pro Pro Ala
            500             505                 510
Arg Gly Pro Pro Pro Pro Pro Trp Asn Cys Trp Pro Pro
            515             520
```

```
<210> 16
<211> 846
<212> DNA
<213> Homo sapiens

<220> -
<223> 866885

<400> 16
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tcaaattgcc caagaaattg ctgagaaaat tcaacaacga aatcaatatg aaagaaaagg 120
tgaaaggca ccaaagctta ccgtgacaat cagagctttg ttgcagaacc tgaaggaaaa 180
gatcgccctt ttgaaggact tattgctaag agctgtgtca acacatcaga taacacagct 240
tgaaggggac cgaagacaga acctcttgga tgatcttgta actcgagaga gactacttct 300
ggcatccttt aagaatgagg gtgccgaacc agatctaatc aggtccagcc tgatgagtga 360
agaggctaag cgaggagcac ccaacccttg gctctttgag gagccagagg agaccagagg 420
cttgggtttt gatgaaatcc ggcaacagca gcagaaaatt atccaagaac aggatgcagg 480
ccttgatgcc ctttcctcta tcataagtcg ccaaaaacaa atggggcagg aaattgggaa 540
tgaattggat gaacaaaatg agataattga cgaccttgcc aacctagtgg agaacacaga 600
tgaaaaactt cgcaatgaaa ccaggcgggt aaacatggtg gacagaaagt cagcctcttg 660
tgggatgatc atggtgattt tactgctgct tgtggctatc gtggttgttg cagtctggcc 720
gaccaactga tggcagtaaa gagaccacca gcagtgacac ctggcaatga cagatgcaag 780
cccaacaccc ttttggtacg caaaacctgc tctcaataaa ttcccccaaa gctctgaaaa 840
aaaaaa                                                             846
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<210> 17
<211> 1897
<212> DNA
<213> Homo sapiens

<220> -
<223> 1273453

<400> 17
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aagtgacatg gactgtgtag tcaccctaac cactgacgct gcacgtcgta tctatgatga   120
aacccaaggt cgtcagcagg tgttgcccct tgattctatt tacaagaaga ctcttccaga   180
ttggaaaaga tctctacctc atttccgaaa tggaaaattg tattttaaac ccattggaga   240
tccagtcttt gctcgagact tgttaacatt tccagataat gtagaacatt gtgaaacagt   300
atttggtatg ctgttaggag acaccattat tttggataat ctggatgcgg ccaatcatta   360
tagaaaagag gttgttaaaa ttacacactg tcctacactg ctgaccagag atggagatcg   420
aattcgaagt aatggaaagt ttgggggcct tcagaataaa gctcctccaa tggataaact   480
tcggggaatg gtatttggag ctccagttcc aaaacagtgt ctgatcttag gggaacaaat   540
agatcttctt cagcagtatc gttctgctgt gtgcaaacta gacagtgtga ataaggatct   600
taacagtcaa ttagagtacc ttcgcactcc ggatatgagg aagaaaaagc aagaacttga   660
tgaacatgag aaaaatctca aactaataga ggaaaaacta ggtatgactc ccatacgtaa   720
gtgtaatgac tcattgcgtc attcaccaaa ggttgagacg acagattgtc cagttcctcc   780
taaaagaatg agacgagaag ctacaagaca aaataggatt ataaccaaaa cagatgtatg   840
agaggtgaca gagagaagag gccattggtc tcagtaagaa tgccctgctt tctgcatctc   900
tgtttcagaa gaccaagagg gtgacttacc agactagta tttctgggga caatacaagt   960
acctgggcat gaatttccat ttcgattcag atgggactgg aaacaaccat tcaattttat 1020
gaatcttact ggacattatg gatttactgg aattattcca gacattatgc cctttggttg 1080
tcactacctt gcaaatgtgt aagaggaaaa tgtgctaatg tggcagtgac tgtaaaactg 1140
gcacatggca tttattaatc ctgaagaaaa gtacatgtac tattttcag tataaatata 1200
atgaacatgt cagaactatt tcttgaaaac cttttatta cttttgcgtg aatttattta 1260
acaaagatgt tttgtctttt gtgtaaggga ggttctagag ctagatgtt taattgtaaa 1320
tatgtgagga aactcaatgc agaattcagg ataaaaattt taaaagcaca ggtatttggg 1380
aattgaaatg ttaagatacc cagaacaaca ttaaatcaat gagtgaactt gtgacagtgg 1440
tagcatttca aatttcaaaa gacttatcct gtgtgtgtgt gtgtgtgtgt atatatatat 1500
atatatatat aaatatatat atataaaata ttcagcagca ccaagtttta taactattgt 1560
ttgtttgact ttattaatac tagaatatgt agtctcagcc ttaattttac atttacatta 1620
ttttgtaatt ttttattact atttttaagg ggttaaagag aacatacatt ctcacattag 1680
tgtactttct ggtagaaagt tgctgcaaaa acatttgaaa tgtatattaa cctaatgtat 1740
gtcatatata tgtctttgtg taagttcaag actattgatc tgtgaagtta ttttgtaagg 1800
acatacattt ggtaagtaag tttgtgtccc aggaaatgta tgtgtttta aacccttct 1860
aaatatgcag gccattaata aataagattg tgtctca   1897

<210> 18
<211> 2272
<212> DNA
<213> Homo sapiens

<220> -
<223> 1534876

<400> 18
ccatgctcca ggcatacaga tgtggtttct cggctgcacc gggccaggct gcgggtgtgc   60
aggcgtctgc aaagttgtgc catgtatcag cacaggcttt gagacgtctg gaccctgtcc   120
ttcctcccgt gaggggttct tgttcttct gactcaggtg acttttcagc ccttccaatt   180
cccctctttt tctgccctcc cctccaactc agccaaccca ggtgtgggca gtcagggagg   240

```
gagggagtgt cccaccacgt tctcagggca gcccttgact cctaagcccc ttcctccttc 300
cattctgcat cccctcccca tccaacctaa atgcccacag ctggggctga gctgtattcc 360
tgtggaggga cctctgccgt gcctctctga ggtcaggctg tgctgtgtga tgggcaggct 420
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gggcccgtcg cttccctgcc agtgaggagt gacttctccc tctcttccag tcctgtaggg 540
gagacaaaac cagattgggg ggcccaaggg gagcatggaa aaggccggct cccctgtctt 600
tccttggctg tcagagtcag ggtaacacac accaagagtg gagtgcggcc agcaagtttg 660
agacctgccc gccctcctcg cagctctgct ctgtgtcctc aggaagtcac agagtctact 720
gaggcaagga gagggtgatt ctttccccaa atcccttctt ccctggttcc caaaccaaag 780
acagcctgca gccctttctg catggggtgc tctgttgaca ggcttcccag atccctgagt 840
ctctctttcc ttcctcctcg atctttagtt gtccacggtc aattcagtgc ttccattggg 900
ggacagtccc ctccgggatg acctgattca cctccagccc agggaatgga atctagagga 960
atacgtgggg tgggtctgga caaggagcgg caggaatcac cacccatctc cagctgtgga 1020
gccctgtgga ggggaagggg aagcttgggg ttcagaggga actcttccag gagaggggtg 1080
cccagcggag gtaaagatga tagagggttg tggggggtct ctagttgaat gttttggccc 1140
atgactttgg aacatggctg gcagcttcca gcagaagtca cgctccccat cccccagggg 1200
acataggacc ttttttcctgc ttcctggtca ctttcaaaga actatttgcg caatctgtgg 1260
gtctgtggat tcacggggct ttctgtgtgg gtgctgcagt tgctttttgtc tgcagcagca 1320
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agccaggtgg ctcatgggct gtagtgagcc agctccctgg gggaaaaggc tgtgggccgt 1500
taggaccatc ctccaggaca ggtgacctct atgaggtcac ctacggctgt ggccgtgcag 1560
gcctccttcc agcccagagt ggcccagtag agcaaggcag acagtgacct ccaccccgc 1620
agccctctta aaaggccagt actcttgggg gtggggggag ggtttagaaa gcatttgccc 1680
atctgccttt ctttcccca gcccccaccc gctttgaatg tagagacccg tgggcacttt 1740
tcctttttgtg gtggggggtg cggaggaggt acccccaccc ctggcacagc cgcctggaat 1800
gcaggactgt cactgctgtt cgggtgatga cctcgttgcc aagctcctcc tgtcccttg 1860
ttctgggggc aggcgctgtg cttctgtgag gtggtttagc ttttgctttc gaagtggcca 1920
gctgcggcca ccaggtctca gcacaagagc gcttcctttg cacagaatga gcttcgagct 1980
ttgttcagac taaatgaatg tatctgggag gggtcggggg cacgagttga ttccaagcac 2040
atgcctttgc tgagtgtgtg tgtgctggga gagtcagagt ggatgtagag cgcggtttta 2100
tttttgtact gacattggta agagactgta tagcatctat ttatttagat gatttatctg 2160
gtaaatgagg caaaaaaatt attaaaaata cattaaagat gatttaaaaa aaagaccaaa 2220
aaaccaagaa acccaaagcc caagaatgcg cgtagcatcc aaaaaaaaaa gg        2272
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<210> 19
<211> 992
<212> DNA
<213> Homo sapiens

<220> -
<223> 1634813

<400> 19
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gacagcttgg cctacagccc ggcgggcatc agctcccttg acccagtgga tatcggtggc   60
cccgttattc gtccaggtgc ccagggagga ggaccgcct gcagcatgaa cctgtggctc  120
ctggcctgcc tggtggccgg cttcctggga gcctgggccc ccgctgtcca cgcccaaggt  180
gtctttgagg actgctgcct ggcctaccac taccccattg ggtgggctgt gctccggcgc  240
gcctggactt accggatcca ggaggtgagc gggagctgca atctgcctgc tgcgatattc  300
tacctcccca agagacacag gaaggtgtgt gggaacccca aaagcaggga ggtgcagaga  360
gccatgaagc tcctggatgc tcgaaataag gttttttgcaa agctccgcca caacacgcag  420
accttccaag caggccctca tgctgtaaag aagttgagtt ctggaaactc caagttatca  480
tcatccaagt ttagcaatcc catcagcagc agcaagagga atgtctccct cctgatatca  540
gctaattcag gactgtgagc cggctcattt ctgggctcca tcggcacagg aggggccgga  600
tctttctccg ataaaaccgt cgccctacag acccagctgt ccccacgcct ctgtcttttg  660
```

```
ggtcaagtct taatccctgc acctgagttg gtcctccctc tgcacccccca ccacctcctg 720
cccgtctggc aactggaaag agggagttgg cctgattta agccttttgc cgctccgggg 780
accagcagca atcctgggca gccagtggct cttgtagaga agacttagga tacctctctc 840
actttctgtt tcttgccgtc caccccgggc catgccagtg tgtccctctg ggtccctcca 900
aaactctggt cagttcaagg atgcccctcc caggctatgc ttttctataa cttttaaata 960
aaccttgggg gttgatggag tcaaaaaaaa aa                              992
```

```
<210> 20
<211> 810
<212> DNA
<213> Homo sapiens

<220> -
<223> 1711840

<400> 20
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tggaggcggg ggtggggggtc tgagctgcgt cctgggctcg aggcgtcccc cggggagtcg 120
cctcttagcg gtgcgtccgg gctagcggcg aggggccgcc ccaagtcttc ccaccgccgc 180
caccttagca gcccgacttg gggcctggaa agtggagcac gcggaggtgg gagggccctg 240
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<211> 1064
<212> DNA
<213> Homo sapiens

<220> -
<223> 1747327

<400> 21
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gacctgcccc ctggcattcc ctggccacgt ttcccaggcc cttgggaccc tcctgttttt 180
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```

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```

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<210> 22
<211> 1336
<212> DNA
<213> Homo sapiens

<220> -
<223> 1864292

<400> 22
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<211> 1742
<212> DNA
<213> Homo sapiens

<220> -
<223> 1866437

<400> 23
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caagaccaga aagctccaga taaagaggcc atactgcggg ccaccgccaa cctgccctcc 480
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<210> 24
<211> 1074
<212> DNA
<213> Homo sapiens

<220>
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<221> unsure
<222> 708, 718, 730, 738, 741, 770, 775, 780, 783, 803, 813, 818,
<221> unsure
<222> 819, 839, 844, 864, 870, 890, 901, 903, 918, 921, 943, 959,
<221> unsure
<222> 960, 967, 975, 990, 997, 999, 1000, 1007, 1040, 1053, 1055,
<221> unsure
<222> 1065, 1071
<223> a or g or c or t, unknown, or other

<220> -
<223> 1871375

<400> 24
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```

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```


<210> 25
<211> 1454
<212> DNA
<213> Homo sapiens

<220> -
<223> 1880830

<400> 25
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ccggacagtg ggagtgcccc tatgatcacc tgccgagtct gccaatctct catcaacgtg 180
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aaaaaaaagg gcgg                                                  1454
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<210> 26
<211> 1121
<212> DNA
<213> Homo sapiens

<220> -
<223> 2328134

<400> 26

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```

```
<210> 27
<211> 1229
<212> DNA
<213> Homo sapiens

<220> -
<223> 2652271

<400> 27
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```

```
<210> 28
<211> 2295
<212> DNA
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<213> Homo sapiens

<220> -
<223> 2965248

<400> 28
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caccatggcc agcaccattt ccgcctacaa ggagaagatg aaggagctgt cggtgctgtc  120
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<210> 29
<211> 2215
<212> DNA
<213> Homo sapiens

<220> -
<223> 3057669

<400> 29

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